normal brain tissues. The Bmi1 and Ezh2 protein levels were also assessed in U-87MG, T-98G, A172 and U118MG glioblastoma cell lines by Western blotting.
**Results:** Bmi1, Ezh2 and nestin were not detectable in normal brain. In contrast Ezh2 and Bmi1 were expressed in most brain cancers. Their expression was generally higher in low-grade astrocytomas than in high-grade astrocytomas and in a case of Ezh2 this difference was statistically significant. Similarly, ependymomas and meningiomas displayed higher expression of Bmi1 and Ezh2 when compared to anaplastic ependymomas and malignant meningiomas. Increased expression of Bmi1 and Ezh2 was also found in oligodendrogliomas and meduloblastoma. However, results in these tumors did not reach statistical significance. In comparison to other cell lines, expression Bmi1 and Ezh2 was markedly higher in U-87MG (anaplastic astrocytoma).
**Conclusion:** We can speculate that increased expression of PcG in low-grade tumors may reflect their ability to keep the neoplastic cells in a more differentiated state which is supported by GFAP positivity of these cells. An alternative explanation is that increased expression of nestin and lower expression of PcG in high-grade tumors could indicate dedifferentiation of tumor cells. Taken together, this study shows that Bmi1 and Ezh2 proteins are differentially expressed in brain tumors and suggests that alteration of these PcG proteins might be one of the events that leads to the development of brain cancer. These findings are practically relevant since both Bmi1 and Ezh2 PcG proteins have recently been suggested as candidates for targeted therapy. Supported by MSM 6198959216, FRVS 1722/2006 and NR 8370/03.

### 686 OLIGODENDROGLIOMAS ARE GLIONEURONAL TUMORS

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**Background:** Conflicting evidence about neuronal differentiation in oligodendroglioma (ODG) exists.
**Design:** We performed an electron microscopic and immunohistochemical (IHC) study of 41 differentiated and anaplastic, frontotemporal ODGs. For the immunohistochemical identification of synaptophysin, monoclonal antibodies (mAbs) clones 27G12, Snp88, and SY38, and a polyclonal antibody (pAb) (A0010, Dako, Glostrup, Denmark) were compared in optimized protocols on slides from multitissue blocks containing a wide spectrum of normal and neoplastic tissues and a multitissue block with 16 ODGs. For the identification of chromogranin, mAbs clone LK2H10 and DAK-A3 and a pAb (A04030, Dako) were compared in a similar way. The best performing synaptophysin and chromogranin Abs were used for staining all ODGs. All cases were stained for synapsin I using mAb clone A10C. FISH analysis for 1p and 19q deletions was carried out on the multitissue block.
**Results:** In all ODGs, tumor cells with neuritic structures were identified ultrastructurally: stubby cytoplasmic processes containing microtubules arranged in parallel arrays, complete or incomplete synapses and sparse neurosecretory granules. For the immunohistochemical identification of synaptophysin, mAb clone 27G12 gave the best signal-to-noise ratio, while SY38 gave markedly less intensely signal than the others, and a negative result in 10/16 cases. When clone 27G12 was applied on all 41 ODGs, a diffuse and/or dot like positive staining reaction was obtained in 100% of the tumors. Among the three chromogranin Abs compared in optimized protocols on the multitissue blocks, clone LK2H10 and pAb A04030 gave identical staining patterns with 3 positive ODGs, while mAb DAK-A3 gave negative reactions in these. When LK2H10 was applied on all 41 ODGs, a diffuse and/or dot like positive staining reaction was obtained in 12 of the tumors (29 %). Synapsin I was detected in all tumors in the large majority of tumor cells. FISH analysis on the multitissue block showed a concomitant 1p/19q deletion in 12/16 ODGs.
**Conclusion:** Our study provided substantial evidence for an extensive neuronal differentiation in ODGs. Optimized immunohistochemical protocols including well performing antibodies are mandatory for positive staining results.

### 687 "POLYMYOSITIS" IN PATIENTS WITH MYASTHENIA GRAVIS AND THYMOMA - RATHER A PARANEOPLASTIC EVENT THAN A REAL PRIMARY AUTOIMMUNE INFLAMMATION

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**Background:** Lymphocytic infiltrate in muscle of patients with myasthenia gravis (MG) and thymoma was reported repeatedly as a coincidental autoimmune polymyositis. In this study we aim at providing evidence that most cases of such a „polymyositis" do not represent a myositis per se, but only a paraneoplastic event.
**Design:** An excision from the sternothyreoid muscle taken during thymectomy was examined histopathologically in 28 patients with diagnosed MG and thymoma. Provided that lymphocytic infiltrates were found, we performed immunohistochemistry to establish their immunophenotype (CD20, CD3, CD4, CD8, TdT, CD68). Further, an antibody against CD45RA antigen (physiologically present in B-cells and mature but naive T-lymphocytes) was applied. We also searched immunohistochemically for the expression of HLA-ABC antigens on the surface of the muscle fibers. The immunophenotype of thymoma-lymphocytes was also analyzed. The findings in muscles were compared with those obtained in 14 patients with definite polymyositis.
**Results:** In all polymyositis cases, the inflammatory infiltrate was composed mostly of CD8+CD4- T-lymphocytes and scattered macrophages and there was a strong diffuse expression of HLA-ABC antigens found on the surface of muscle fibers. All the CD8+ cells in polymyositis were CD45RA negative. In 16 patients with MG and thymoma the lymphocytic infiltrates were identified in muscles and they were morphologically indistinguishable from those in polymyositis. However, the expression of HLA-ABC was limited to the muscle fibers close to the lymphocytic infiltrates, all other fibers were negative. The lymphocytes were CD8 positive, but a small proportion of them co-expressed the CD4 antigen. The CD8+ T-lymphocytes were simultaneously CD45RA positive. The thymic tumors of the 16 patients were all thymomas of type B or AB. No lymphocytes were found in muscles in MG patients with type A thymoma. Although most of the intratumoral lymphocytes were TdT+ immature T-cells, we have identified a proportion of CD8+CD45RA+ cells admixed to the thymocyte-population. Clinically, the MG patients with the lymphocytic infiltrates in muscle did not differ significantly (both in the preoperative presentation and in the follow-up analysis) from those without the infiltrates. In both groups, the muscle weakness improved after the thymoma removal.
**Conclusion:** It has been shown previously that the proportion of CD8+CD45RA+ lymphocytes is significantly increased in the blood of patients with thymomas (Hoffacker et al., 2000). We identified CD8+CD45RA+ T-cells in thymomas and demonstrated that the cells of the polymyositis-like lymphocytic infiltrates in muscles have the same immunophenotype, different from that of polymyositis. Therefore, we suggest that the lymphocytic infiltrates in patients with MG and thymoma represent more likely a paraneoplastic event due to the "spillover" of thymoma-derived mature naive T-cells than a real cell-mediated autoimmune disorder. The finding CD8+ CD45RA+ lymphocytes in muscle biopsies (especially in MG patients) should not be interpreted as a polymyositis, but should lead to the exclusion of an underlying thymic neoplasm. Supported by: IGA MZCR NR/8924-3 and VZ FNM 00064203.

### 688 TOXIC NEUROLOGIC EFFECTS OF RADIATION: A CASE REPORT OF UNDETERMINED ETIOLOGY

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**Background:** Radiation necrosis of the brain is acknowledged as a long term side effect of radiation therapy to the head and neck. Histologic features consistent with a diagnosis of radiation necrosis include edema, microcalcifications and confluent coagulation necrosis without peripheral pseudopalisading. Scattered macrophages may be present. Degenerative vascular changes, i.e. fibrinoid necrosis, telangiectases, and mural hyalinization, are seen in lieu of true vasoproliferation.
**Design:** This is a case report of a 45 year old Caucasian man who presented to the emergency department complaining of left sided headaches, nausea and vomiting for three weeks associated with mental status changes.This patient is a married man who works in the quality control division of a nuclear plant. His social and medical history are non-contributory. He has no history of radiation therapy. His mother died due to complications of multiple sclerosis. Physical examination revealed right sided facial, difficulties with right sided hand-eye coordination, gait imbalances and a positive Romberg's sign. Imaging studies of his brain revealed a left frontoparietal mass surrounded with left frontal edema and a midline shift, consistent with a primary brain tumor such as glioblastoma multiforme, lower grade astrocytoma or, less likely, metastatic disease or a brain abscess. Metastatic workup was negative. Based on history and physical examination findings, the patient and surgeon agreed on surgical excision of the lesion. Pathologic Findings: Grossly, the specimen was composed of multiple fragments of soft tissue aggregating to 3cm in greatest dimension. The permanent microscopic sections demonstrated predominantly necrotic brain parenchyma with hyalinized blood vessels, fibrin thrombi and perivascular lymphocytic infiltrate. The preserved parenchyma was edematous and hypercellular with atypical and occasional multi-nucleated astrocytes. Astrocytic gliosis and foamy macrophages were also seen. Additionally, a focal area of calcification was present. These findings were consistent with a pathologic diagnosis of radiation necrosis. The differential diagnosis for atypical astrogliosis includes astrocytomas; however, there was no histologic evidence of malignancy.
**Conclusion:** It is commonly understood that radiation exposure above 50 grays can result in radiation necrosis of the brain. It is also known that the effects of exposure do not depend solely on the total dose but the fractionization and timing of the doses and that this total dose may actually be lower that what is currently considered clinically acceptable. In these cases, the exposures have all been a result of focused radiotherapy to the head and/or neck. We have learned from the atomic bomb exposures in Hiroshima and Nagasaki that the estimated lethal dose of whole body radiation for 50% of the population is somewhere between 3 and 4 grays. According to the United States Nuclear Regulatory Commission, the total effective occupational dose limit of radiation for adults is 0.05 grays. The USNRC also states that the total dose committed to any particular organ or tissue is 0.5 grays (except for the eye, which is 0.15 gray). At these doses, it has not been reported that human brains will undergo radiation necrosis. However, our patient has no other exposures to radiation that can account for his brain lesion.

## Ophthalmic

### 689 ORBITAL INVOLVEMENT IN ANGIOLYMPHOID HYPERPLASIA WITH EOSINOPHILIA

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**Background:** Angiolymphoid hyperplasia with eosinophilia (ALHE) and Kimura's Disease (KD) share many clinical and histopathological features. Although they were once considered different stages of the same disease, they are now known to be separate entities. The former is a localized hyperplasia of atypical endothelial cells with no systemic

involvement. On the other hand, the latter can course with lymphadenopathy, blood eosinophilia and nephrotic syndrome due to IgE deposition in the renal glomeruli.
**Design:** Case report.
**Results:** Clinical- An eighteen year-old asian female presented to the ophthalmology clinic of the McGill University Health Centre with a three-month history of fluctuating swelling and ptosis of the left upper eyelid. There was also mild discomfort associated whenever the swelling was more intense. A well-defined, soft lesion in the left upper lid could be palpated, just below the superior orbital rim. No decrease in visual acuity or alterations of extraocular movements were found. Intraocular pressure was slightly higher in the affected side. Computed tomography disclosed a distinct homogeneous orbital lesion in the left superior orbit, molding to the globe and other orbital structures. There was no bone erosion. The findings favored the diagnosis of a lymphoid lesion and a transpalpebral biopsy was indicated and performed. Pathology- Histopathological evaluation revealed the presence of structures resembling lymphoid follicles surrounded by loose connective tissue. At higher magnification, those structures were composed of numerous blood vessels lined by plump endothelial cells with oval nuclei protruding into the lumen. Surrounding the vessels, there was a chronic inflammatory infiltrate composed of lymphocytes, plasma cells and a large proportion of eosinophils. Immunohistochemistry with Factor VIII was done and highlighted the prominent vascular component of the lesion. The "epithelioid" or "histiocytoid" atypical cells all showed positive immunostaining. Whole body gallium scan failed to reveal lymph node involvement elsewhere. Blood counts and urinalysis were also normal. Based on clinical and histopathological findings, the diagnosis of ALHE was made.
**Conclusion:** Although exams like blood count, urinalysis and whole body scans can assist in the differential diagnosis, ALHE can be diagnosed and differentiated from KD on histopathological grounds. The presence of vascular hyperplasia with plump endothelial cells protruding into the lumen is the most important feature in establishing the diagnosis of ALHE. Such differentiation is crucial for the patient because ALHE is not associated with any of the systemic manifestations present in KD.

### 690 LIPOMATOUS HAMARTOMA OF THE ORBIT. CASE REPORT AND LITERATURE REVIEW

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**Background:** Proliferation of mature adipose tissue rarely occurs in the orbit. Lipomatous hamartoma (LH) is a rare benign condition that is reported in several sites. We acknowledge only one previous case of LH reported in the orbit.
**Design:** We report the second case of orbital LH.
**Results:** A 45 year-old man presented with proptosis to the ophthalmologist complaining about decreasing visual acuity for several months. A CT scan showed a non-encapsulated orbital mass located in the posterior pole near the optic nerve. The mass was totally resected. Histopathology showed a non-encapsulated irregular tumor composed mainly of mature adipose tissue with regularly sized adipocytes. Fibrous connective tissue forming fibrous septa, and abnormal sized blood vessels were commonly seen throughout the lesion, associated with "follicle-like" aggregates of lymphocytes. Abnormal thick nerve bundles were also present. The diagnosis of LH of the orbit was made. The patient was free of recurrence within 6 months of follow-up.
**Conclusion:** LH is a benign tumor with high recurrence rate, which can be distinguished clinically & histopathologically from dermolipomas, herniated fat, lipomas and liposarcomas. We have described the second case of LH of the orbit.

### 691 EYELID TUMORS: CLINICOPATHOLOGICAL CORRELATION OF 1334 CASES

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**Background:** To study epidemiological, clinical and pathological aspects in a series of eyelid tumors, and to compare benign and malignant tumors for those variables. To determine the clinical diagnostic sensitivity and specificity, and the pathological base of ocular cutaneous horns.
**Design:** 1334 eyelid tumors were processed and paraffin embedded, and light microscopic study was made. Epidemiological and clinical aspects (sex, age, localization and size of the lesions, clinical diagnosis) were also collected. Statistical analysis was made, by comparison of percentages and Chi square test.
**Results:** Benign tumors were 68% of the total, 57% being of epithelial origin. The most frequent benign tumor was the seborrheic keratosis and the most frequent malignant one was the basal cell carcinoma (the most frequent tumor in total). Benign tumors were mostly located in the upper eyelid, while malignant tumors predominated in lower eyelid and internal canthus. Size was larger in malignant tumors. There is a statistical correlation between older ages and the development of malignant tumors (and of course between childhood and benign tumors). The pathological bases of cutaneous horns were verruca vulgaris, actinic keratosis and seborrheic keratosis. None of the cutaneous horns had a malignant base. Accuracy in the diagnosis showed a sensitivity of 83% and a specificity of 88%.
**Conclusion:** Frequency of eyelid tumors received in our pathology laboratories has increased during the last ten years. There is a predominance of benign tumors and most of them are epithelial, followed in order by the adnexal and melanocytic ones. Sensitivity and specificity for clinical diagnosis is acceptable.

## Oral

### 692 DETECTION OF EPSTEIN-BARR VIRUS (EBV) BY IN SITU HIBRIDIZATION IN LESIONS LIKE ORAL HAIRY LEUKOPLAKIA

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**Background:** Epstein-Barr virus (EBV) is a human herpesvirus that estabilishes persistent infection and is associated with many diseases, including infectious mononucleosis syndrome,lymphomas, nasopharyngeal carcinoma, and oral hairy leukoplakia, affecting principally immuno- compromised patients. Oral hairy leukoplakia is a non malignant, EBV-associated, epithelial disease that typically occurs on the lateral tongue borders. It is common in individuals with HIV infection and in patients receiving iatrogenic immunossupression. Histologically, hairy leukoplakia is characterized by shaggy hyperparakeratosis, acanthosis, "koilocyte"-like or balloon cells, and a paucity of inflamation. The histologically features of hairy leukoplakia are not pathognomonic, and for the many authors definitive diagnosis requires demonstration of EBV.
**Design:** The aims of this study were to verify the presence of EBV, by in situ hibridization in lesions diagnosed histologically suggestive of hairy leukoplakia and compare this results with histologically features.
**Results:** Thirty six biopsy specimens from lesions histologically suggestive of hairy leukoplakia were selected from the Department of Stomatology's Oral Pathology Service archives. EBV in situ hibridization was performed on all 36 cases, and 27 cases (75%) were positive, confirming the diagnose of oral hairy leukoplakia. Histopathologic features did not agree well with EBV in situ hibridization.
**Conclusions:** We concluded that H&E histopathology should not be used as a substitute for in situ hibridization in the definitive diagnosis of hairy leukoplakia.

### 693 USP6 ONCOGENE IS EXPRESSED IN ANEURYSMAL BONE CYSTS OF THE JAWS BUT NOT CENTRAL GIANT CELL GRANUMOMAS

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**Background:** Several chromosomal translocations which upregulate the USP6 oncogene have been recently identified in primary aneurysmal bone cysts (ABC). The vast majority of these cases were from extragnathic bones. Primary ABC of the jaws is a rare lesion that shares histopathologic features with central giant cell granuloma (CGCG), although clinical manifestations may be distinctively different. The aim of this study was to determine if USP6 protein is overexpressed in ABCs and CGCG of the jaws.
**Design:** Twelve cases of CGCG, five cases of ABC and seven cases of peripheral giant cell granuloma, all of the jaws were retrieved from the UCSF Oral Pathology files. In addition, four cases of ABC of extragnathic bones were retrieved from the files of the UCSF Department of Pathology. After review and verification of the diagnoses, paraffin embedded tissue sections were immunostained using an antibody for USP6 (ABCAM; 1:50). RNA extracted from paraffin embedded tissues was reverse transcribed to cDNA and a nested PCR method analysis was used to identify the chromosomal translocation CDH11-USP6.
**Results:** Four of five ABCs of the jaws and three of four ABCs of the extragnathic bones showed cytoplasmic expression of USP6 protein but none of the twelve cases of the CGCGs were positive. Using PCR analysis we found that one ABC showed the CDH11-USP6 chromosomal translocation that upregulates USP6 protein expression.
**Conclusion:** We show that a majority of aneurysmal bones cysts of both the jaws and extragnathic bones overexpress USP6 protein. Overexpression was not seen in any of the CGCGs and immunostaining for this oncogene may be helpful in differentiating this from ABC. We were also able to identify a specific chromosomal translocation in ABC involving USP6 and CDH11 that leads to protein overexpression. This study supports the notion that ABC is a neoplasm associated with upregulation of the USP6 oncogene while CGCG is not and that these two lesions are distinct biologic entities.

### 694 NICOTINE-INDUCED CHEMORESISTANCE IN HEAD AND NECK SQUAMOUS CELL CARCINOMA AND STRATEGY FOR OVERCOMING RESISTANCE - ON THE BASIS OF KB CELL LINE AND BETULINIC ACID

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**Background:** Nicotine is an important component in cigarette smoke that can activate growth-promoting pathways to facilitate the oncogenesis of various organs. Moreover, growing evidence suggests that cigarette smoke can affect the responsiveness of cancer cells to treatment, especially those of head and neck cancer. The present study describes the effects of nicotine on the cell death pathway, resulting in a decreased cytotoxicity of anticancer drugs such as cisplatin and etoposide. And also it describes the effect of betulinic acid as a chemosensitizer.
**Design:** The author assessed the apoptotic pathway after treatment with nicotine (200 nM/L), and compared the effect of anticancer drugs such as cisplatin (IC50=48 ÿg/ml) and etoposide (IC50=13 ÿg/ml) in the presence or absence of nicotine in oral squamous cell carcinoma (SCC) KB cell line. Also evaluated was the combination chemotherapeutic effect of betulinic acid with well-known chemotherapeutics (cisplatin: IC30=23 ÿg/ml, etoposide: IC30=8.3 ÿg/ml) in the presence or absence of nicotine.
**Results:** Nicotine induces poor phosphorylation in association with suppression of apoptosis in KB cells (inhibition rate: -20±1%). The inhibition rates of KB cells co-treated with anticancer drugs and nicotine were significantly decreased (cisplatin: 31±2%, etoposide: 24±1%) comparing to anticancer drug only treated group (cisplatin: 41±1%, etoposide: 35±1%) ($P<0.01$). However, chemoresistance caused by nicotine was subjugated by combination of betulinic acid (16 ÿg/ml) and chemotherapeutics (cisplatin: 56±2%, etoposide: 62±2%) ($P<0.001$).