symptoms related to his hypopharyngeal cancer. Also, his chief complaint was not directly related to his primary cancer and it was only at the time of autopsy that the hypopharyngeal mass was identified; 2) To our best knowledge, this is the heaviest liver ever reported for any primary or metastatic cancer. The patient did not have any risk factor for liver disease and although he had the history of alcohol abuse, there was no histologic evidence of cirrhosis in the liver in postmortem examination. Primary clinical presentation and ultimate cause of death were all attributed to metastatic squamous cell carcinoma with hypopharyngeal cancer as the primary origin.

24 A Case of Pulmonary Zygomycosis Associated with Calcium Oxalate Deposition within Bronchial Cartilage

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Background: The deposition of calcium oxalate crystals associated with fungal infection, especially, *Aspergillus niger* is well known and is considered an important diagnostic feature. It is seen in pulmonary and extra-pulmonary sites. Crystals of calcium oxalate are thought to be formed from oxalic acid produced by the fungus and calcium present in the tissue. Thus, they are usually seen at site of infection. Oxalic acid may cause local tissue damage and vascular involvement may cause hemorrhage. We report a case of pulmonary zygomycosis with calcium carbonate and calcium oxalate crystal deposition, the latter showing unusual involvement of bronchial cartilage.

Design: We reviewed the clinical and pathological results from an autopsy case of pulmonary zygomycosis. Lung tissue was examined by light microscopy, scanning electron microscopy with energy dispersive X-ray analysis (SEM/EDXA), and infrared spectroscopy (IR).

Results: A 75 year old white female with acute onset of shortness of breath, cough, and hemoptysis was treated for community acquired pneumonia, but despite intensive therapy had an unfavorable hospital course and expired 16 days after admission. An autopsy revealed multifocal necrotizing pneumonia associated with mixed herpes virus and fungal infection, and diffuse alveolar damage. Fungal elements displayed broad-based, branching, hyaline, septate hyphae consistent with *Zygomycetes spp*. Fungal lung culture grew *Rhizopus spp*. Brightly birefringent particles were seen in association with fungi. Unusually, some of the particles were present within bronchial cartilage. These particles stained with Von-Kossa but not with alizarin red, consistent with calcium oxalate. SEM/EDXA mapping demonstrated areas of calcium, oxygen, (and carbon) collocation within the cartilage. By infrared spectroscopy, birefringent crystals in the bronchial cartilage had the spectral characteristic of calcium oxalate monohydrate. Calcium oxalate dihydrate was identified in other locations, and calcium carbonate was present in fungus-invaded vascular walls.

Conclusions: This case documents an unusual association of pulmonary zygomycosis with tissue deposition of calcium oxalate, where the calcium oxalate crystals were deposited within bronchial cartilage. As the fungus infected tissue is the presumed source of the oxalate, it may be that the availability of calcium facilitates crystal deposition within the bronchial cartilage.

25 Clinical Setting Does Not Predict Discrepancy between Clinical and Autopsy Diagnoses

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Background: Autopsy rates in the United States and worldwide have continued to decrease in recent years. A number of factors, including financial concerns, fears of malpractice litigation, difficulty and inexperience in approaching families and advancing diagnostic technology have resulted in a cultural change in Medicine regarding the autopsy. Despite this, a number of important studies have shown that the discrepancy rate between clinical diagnoses and postmortem findings has not changed significantly. (Goldman, 1983) (Vincent, 2004).

Design: The purpose of this study was to determine whether the discrepancy rate between clinical and autopsy diagnoses differed between patients that had either (1) the benefit of intensive medical evaluation, (2) those that underwent recent surgery, and (3) those that did not have the benefit of such evaluation. A retrospective comparison of clinically and post-mortem determined primary cause of death and level of discrepancy for patients treated in the medical intensive care unit (MICU), patients that had undergone recent surgical procedures (SURG) and patients autopsied from affiliate nursing homes (NH) was made. Associations between diagnostic discrepancy and age, sex, and year of diagnosis were also examined.

Results: From 2003 to 2006, 562 adult autopsies were performed at our institution. Of these, 285 expired in one of the 3 clinical settings considered in this study (MICU:108, SURG:97, NH:80). The overall diagnostic discrepancy rate was 27% and was not significantly different between the 3 groups (p=0.828). Diagnostic errors were classified in two broad categories according to criteria established by Goldman, et al. Class I discrepancies included missed major diagnoses that, if detected prior to death, could have influenced management and might have resulted in prolonged survival. Class II discrepancies included major diagnoses that would not have altered management. Of the discrepancies, 65% were Class I and 35% were Class II and this did not vary between groups (p=0.728). Diagnostic discrepancy was also not associated with age (p=0.265), sex (p=0.425), or year of expiration (p=0.937).

Conclusions: Discrepancy rates of postmortem diagnoses and antemortem clinical diagnoses did not differ significantly among the three patient populations and were independent of clinical setting, level of antemortem evaluation, age, sex and year of expiration.

26 Prevalence of Respiratory Pathology in a Cohort of 784 Adult Autopsies

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Background: Respiratory pathology findings are said to be common in autopsy populations but their nature and prevalence are not well documented. The purpose of this study was two fold: First, to ascertain the overall prevalence of respiratory disease in a large cohort of autopsied patients and second, to investigate the possible impact of newer diagnostic technologies (ie, spiral chest ct) and therapies (ie, last generation antimicrobials) on the mortality of a subset of patients, whose deaths were directly attributable to pneumonia.

Design: We reviewed the autopsy protocols of 1107 autopsies performed over a period of 10 years. Autopsies performed in fetuses and children (n199) and restricted autopsies (such as heart only) (n124) were excluded, leaving a pool of 784 cases; 440 men, 344 women, mean age 62 years. In this pool, respiratory disease was categorized according to major etiologies (ie, malignancy, pneumonia, thromboemboli). For comparative analysis, patients identified as dying primarily of pneumonia were divided into 2 consecutive five year periods.

Results: Respiratory abnormalities were found in 703 (89.6%) of the 784 autopsies. Of the 703 cases, 191 had pleural abnormalities and 17 were tracheobronchial cases. The remaining 475 cases had parenchymal lung disease with pneumonia (n 241), thromboemboli (n 144), malignant neoplasms (n 93) and diffuse alveolar damage (n 91); as the leading disease entities. Of the 241 cases with of pneumonia, 204 had comprehensive documentation and of those 71 (34.8%) had deaths directly attributable to pneumonia. In 133 cases, pneumonia was interpreted as a major co-morbidity but not directly associated with death. Among the 71 patients with deaths attributable to pneumonia, 32 occurred during the first 5 year period and 39 during the second 5 year period (p=0.3132 by Poisson test).

Conclusions: This study documents an extraordinarily high prevalence of respiratory disease among a large cohort of adult autopsied patients. Among the various diagnostic groups, pneumonia represented the single largest group of parenchymal lung disease. Our findings re-affirm the notion that pneumonia ranks highly as a major cause of morbidity and mortality in hospitalized patients. Newer diagnostic technologies and/or therapies appeared to have had no significant impact when pneumonia mortality data for the two 5 year study periods were compared with one another.

27 The Decline of Clinical Autopsy Severely Impairs Healthcare Quality Assurance

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Background: Clinical autopsy provides the basis for education of medical professionals, recognition of environmental influences, infections and occupational toxicity, validation and reliability control of morbidity and mortality statistics. In addition, it is crucial for assessment of diagnostic procedures and therapy, as well as recognition of unexpected complications. Despite the progress of diagnostic technology, the discrepancies between clinical and autopsy diagnosis are acknowledged in medical literature. Our object is to evaluate the status quo of clinical autopsies and the consequences of negligence of postmortem studies on healthcare.

Design: Clinical autopsy data from multiple countries are reviewed. Autopsy frequencies are compared between countries and it tendency is assessed. The discrepancies between clinical and autopsy diagnosis is analyzed. The relationship between medical error rate and autopsy frequency is investigated.

Results: The frequency of autopsies in Germany decreased within the ten year period of 1985 to 1995 from 5.6% to about 1% of all patients deceased in hospital. During the five year period of 1995 to 1999, an additional reduction by 10.7% - 51.3% was registered in the individual German states. Similar trends are noted in the United States and in other Western countries from 1970's to 2004. Cancer, myocardial infarction, strokes, pulmonary embolism, endocarditis, peritonitis, vascular insufficiency of the bowel, lung abscess, duodenal or gastric ulcer, aortic aneurysm and various infections were among frequently missed diagnoses. Fifty eight percent of infectious diseases of adults at a community hospital in the USA were unknown before death. The average frequency of major errors in clinical diagnoses has increased during the same time period. Opposite developments have only been reported from Zurich, Switzerland, where the autopsy frequencies remained at a high level of about 90% of patients deceased in the hospital, and clinical error rates were reduced to about one third.

Conclusions: Autopsy numbers of patients deceased in hospitals have dropped to record lows in Western countries, which severely interfere with the quality evaluation of healthcare. Clinical autopsy discovers missed clinical diagnosis and provides crucial assessment of healthcare quality assurance. In order to keep healthcare at high standards, clinical necropsy ought to be a standard procedure at this time of highly specialized diagnostics and therapy.

Bone & Soft Tissue

28 *BRAF* Mutations Are Present in a Subset of Small Bowel Gastrointestinal Stromal Tumors (GISTs)

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Background: Although most GISTs show activating mutations in either *KIT* or *PDGFRA*, about 10% of cases have a wild-type genotype for these genes. Recently *KIT* mutations have been described in certain subsets of melanomas, which more commonly harbor *BRAF* mutations concentrated in two hot spots, exons' 11 and 15. It

is our hypothesis that activating *BRAF* mutations may also occur in GIST and in this study we screen *KIT / PDGFRA* wild-type GISTs for *BRAF* mutations.
Design: Seventy five GIST patients (51 adults older than age of 30, 7 young adults and 17 children) lacking *KIT / PDGFRA* mutations were identified for the study. PCR amplification from genomic DNA, extracted either from frozen or archival material, was performed for *BRAF* hot spots exons' 11 and 15. The products were screened for mutations using D-HPLC and, in more than half, were confirmed by direct sequencing.
Results: Three (4 %) of 75 *KIT / PDGFRA* wild-type GISTs showed a *BRAF* exon 15 V600E mutation, while none were detected in *BRAF* exon 11. The 3 *BRAF*-mutation positive patients shared similar clinical features, being all females with an age range of 49-55 yrs and having their tumors located in the small bowel. Morphologically all were spindle cell type, had a high risk of malignancy and were immunoreactive for KIT. One patient, who developed liver and peritoneal metastasis, died of disease 18 months following diagnosis and the remaining 2 patients show no evidence of disease at 9 and 13 months.
Conclusions: We identified a *BRAF* V600E mutation in 5.9 % of adult GIST patients, lacking *KIT / PDGFRA* mutations. The *BRAF*-mutated GISTs show predilection for small bowel location, spindle cell phenotype and a high risk of malignancy. Pediatric and young adult GISTs do not appear to harbor *BRAF* mutations. Kinase inhibitors targeting BRAF could be effective therapeutic options in these *BRAF* mutated GISTs.

29 Liposarcoma in Children and Adolescents: A Study of 38 Cases

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Background: Malignant adipocytic tumors in childhood and adolescence are extremely rare, accounting for less than 3% of all pediatric sarcomas. We retrospectively investigated a series of LPS occurring in the first 2 decades in order to assess their morphologic spectrum and its prognostic significance.
Design: H&E stained sections from 38 LPS occurring in patients <21 years of age were reviewed and classified according to the WHO classification. FNCLCC grading was performed. Clinicopathologic information including outcome was retrieved from available medical records in 28 cases.
Results: The results are summarized in the Table. Age at diagnosis was 9 to 21 years; male:female ratio was 2:1. Mean follow up was 3.2 yrs (range 0.6-10 yrs). MLPS accounted for 81% of cases. Five had a round cell component of <5%; one had a >5% round cell component. One of 2 WDLPS had inflammatory features. Among the PLPS, 4 showed an elaborate capillary vasculature, identical to typical MLPS, but exhibited greater cellular pleomorphism, including pleomorphic lipoblasts. In addition, 1 of these cases showed apparent dedifferentiation, with an abrupt transition to areas of undifferentiated sarcoma. One PLPS showed more typical features, with sheets of pleomorphic lipoblasts and necrosis.

Histotype	Age (years)	Site	FNCLCC-Grade	Follow-up
MLPS (31)	14.6 mean (range 9-21)	Head (7%), trunk (43%), extremities (50%)	G1: (30); G2 (1)	No evidence of disease (90%); local recurrence (10%)
WDLPS (2)	15.0 mean (range11-19)	Extremity (100%)	G1 (2)	No evidence of disease (100%)
PLPS (5)	15.8 mean (range 12-18)	Head (20%), trunk (40%), extremities (40%)	G2 (3); G2 (2)	Dead of disease(80%); no evidence of disease (20%)

Conclusions: In sharp contrast to adults, in whom WDLPS comprise most LPS, the great majority (82%) of liposarcomas in the first 2 decades of life are MLPS. Pediatric MLPS may have a more favorable prognosis than do MLPS in older patients, a certain percentage of which will metastasize, even without a round cell component. Interestingly, PLPS were over-represented in the first 2 decades of life, accounding for 13% of cases. As in adults, pediatric PLPS is a highly aggressive sarcoma, frequently resulting in death from disease. The histologic features of childhood PLPS are somewhat different than that of most adult PLPS, often showing a vascular pattern identical to MLPS. On-going genetic study should help to clarify the relationship between childhood PLPS and MLPS.

30 Chondromyxoid Fibroma of Rib with a Novel Chromosomal Translocation: Report of Three Cases at Unusual Sites

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Background: Chondromyxoid fibromas (CMFs) are rare benign chondroid producing tumors that occur in long tubular bones and rarely in small bones of hands and feet. Flat bone involvement is even more uncommon. Prior cytogenetic analyses have identified complex abnormalities involving chromosome 6 in majority of cases.
Design: A search for CMF over 8-year period (1999-2006) from the surgical pathology files of our institution yielded 15 cases. Three cases (14-year old female, 43-year old female and 55 year old male) occurred in relatively unusual regions, 2 from metatarsals and 1 from rib. The rib lesion was resected and the other 2 were curetted. The rib lesion was submitted for routine cytogenetic analysis.
Results: Radiographic studies revealed that all 3 lesions were well-defined expansile radiolucent masses with lobulated margins, sclerotic rim, septation, and no calcification. Grossly, they measured between 1.3 and 6.1 cm in greatest dimension (mean 3.3 cm) and expanded the bony cortices. Cystic changes were present. On histological examination of all 3 lesions, the lobules had "zoned" areas with more cellular peripheral areas mingled with few osteoclast-like giant cells. The central areas were myxoid and less cellular. True hyaline cartilage was not found. The lobules of tumor were present in intertrabecular spaces but lacked an infiltrative pattern of growth. No mitotic figures were detected. No calcification or osteoid was found. With immunohistochemical stains, all 3 lesions were negative for S-100, AE1-AE3, epithelial membrane antigen (EMA) and CD34. Smooth muscle actin (SMA) was positive in 1 of the 3 lesions, and all 3 neoplasms showed low Ki-67 proliferative index of less than 10%. Cytogenetic analysis of rib lesion revealed a novel chromosomal translocation t(1;5)(p13;p13). Although structural aberrations involving bands 1p13 and 5p13 have been described in chondrosarcomas, these have not been previously reported in CMF. None of the 3 patients had a recurrence after a mean duration of follow-up of 23.3 months.
Conclusions: We describe histological findings from CMF originating in unusual locations. These lesions should be distinguished from chondrosarcomas, especially on small biopsies, and should be included in the differential diagnosis. To our knowledge, this is the first report describing a novel chromosomal translocation t(1;5)(p13;p13) in CMF.

31 Aberrant Expression of Epithelial and Neuroendocrine Markers in Alveolar Rhabdomyosarcomas (ARMS): A Potentially Serious Diagnostic Pitfall

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Background: ARMS may be extremely difficult to distinguish from other primitive round cell neoplasms without ancillary immunohistochemistry and/or genetic study. Particularly in adults and in head/neck locations, the differential diagnosis of ARMS includes small cell carcinoma and neuroepithelial tumors, such as esthesioneuroblastoma. We have recently seen cases of genetically confirmed ARMS which were misdiagnosed owing to expression of cytokeratins (CK) and neuroendocrine (NE) markers. We studied a large group of well-characterized ARMS for expression of such markers.
Design: 48 ARMS (22 genetically confirmed) were retrieved from our archives and immunostained for wide spectrum CK (WSCK, OSCAR), low molecular weight CK (LMWCK, Cam5.2), synaptophysin (SYN), chromogranin A (CG), and CD56 using commercially available antibodies. Cases were scored as "negative", "rare" (<5%), "1+" (5-25%), "2+" (6-50%) and "3+" (>51%).
Results: The tumors occurred equally in both sexes (24M, 24F) at a mean age of 18.6 years (range, <1- 64 years), and involved many sites. The IHC results are summarized in the Table. 41% of cases expressed SYN or CG and 10% expressed both. 31% of cases expressed WSCK and SYN or CG. Expression of SYN and CG was typically confined to rare cells but could be more widespread. CD56 expression was near-ubiquitous.

IHC Results

Score	WSCK	LMWCK	CG	SYN	CD56
Negative	23	15	31	27	1
Rare	5	4	5	9	0
1+	9	7	3	3	1
2+	10	4	0	0	4
3+	1	0	0	1	28
Overall (+)	25/48 (52%)	15/30 (50%)	8/39 (21%)	13/40 (33%)	33/34 (97%)

Conclusions: Aberrant expression of epithelial and NE markers is relatively common in ARMS, occuring in 30-40% of cases. These findings have significant implications for the diagnosis of ARMS, particularly in adults and in head/neck locations. Although expression of CK and/or SYN alone does not necessarily indicate epithelial or NE differentiation, co-expression of CK and NE markers, and in particular the presence of CG expression, suggest true epithelial and/or NE differentiation in a subset of ARMS. CD56 is not a specific NE marker, and should not be used in the absence of SYN/CG. These findings emphasize the need to employ a panel of markers, to include desmin, myogenin/MyoD1, and genetic study in the diagnosis of primitive round cell neoplasms in all age groups and in all locations.

32 Patterns and Regulation of HLA Class I Expression in Ewing Sarcoma: Clinical Implications

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Background: Ewing Sarcoma (ES) is the second most common bone sarcoma in children, with a poor prognosis in case of relapse or metastatic disease. *In vitro,* ES are susceptible to T- and, especially, NK cell mediated killing. HLA expression patterns play a crucial role in recognition by tumor-reactive T- and NK cells. This study investigates patterns and regulation of HLA expression in ES cell lines and tumors.
Design: A panel of ES cell lines (n=6), either untreated or pre-treated with IFNγ or epigenetic modulators, were analyzed for HLA expression by FACS, using pan-HLA class I and -II as well as allele-specific antibodies. Moreover, expression of various components of the antigen processing machinery was investigated by Western Blot. In addition, HLA class I and β2-microglobulin (β2M) expression was analyzed by 3-color immunofluorescent staining of a cohort of 75 ES tumors.
Results: ES cell lines show heterogeneous expression patterns for HLA class I alleles, TAP1/2 and tapasin. Pre-treatment with IFNγ, but not epigenetic modulators, induces or increases expression of these molecules, as well as LMP-2 and -7, in all cases. Notably, IFNγ-mediated upregulation of alleles varies among specific alleles and between different cell lines. In 78% of the primary tumors, aberrant expression of HLA class I, as defined as partial or complete loss, is observed, showing inter- as well as intratumoral heterogeneity in β2M and HLA class I expression. 17% of the primary tumors lack β2M expression. Interestingly, all lung metastases lack β2M expression, whereas the bone metastases stain positive for both β2M and HLA class I. HLA class II expression is absent in all cases.
Conclusions: The majority of ES is characterized by aberrant expression of HLA class I. Although this may hamper the interaction with tumor-reactive T cells, it may facilitate possible interactions with tumor-reactive NK cells. Moreover, it may affect prognosis and T-and NK cell infiltration.

33 p16, p53 and EGFR Immunoreactivity Differentiates Osteosarcoma from Benign Tumors of Bone

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Background: Alterations in the Rb and p53 pathways have been implicated in up to 80% of osteosarcomas and EGFR overexpression has been reported in up to 50% of cases. Most studies to date have examined the mechanistic or prognostic significance of these findings; we sought to assess the diagnostic utility of p16, p53 and EGFR in differentiating osteosarcomas from benign osteogenic, spindled or giant cell-rich lesions of bone.

Design: Twenty-four osteosarcomas (18 high grade, 2 parosteal, 2 low grade [mandibular], 2 extraosseous) and thirty-one benign lesions of bone (5 aneurysmal bone cyst, 2 desmoplastic fibroma, 5 giant cell tumor of bone, 4 giant cell tumor of tendon sheath, 6 non-ossifying fibroma, 4 osteoblastoma and 5 osteoid osteoma) with radiographic correlation were immunostained for p16 (BD Biosciences, 1:500), p53 (Dako, 1:50) and EGFR (Dako, 1:200) using a tissue microarray. Strong nuclear staining in >10% of tumor cells for p16 and p53 and strong, complete membranous staining for EGFR (3+) were considered positive.

Results: 63% (15 of 24) osteosarcomas were immunoreactive with antibodies towards p16, p53 or EGFR while only one of 31 benign bone lesions stained (one non-ossifying fibroma, positive for p16 and EGFR). Fisher's exact test analysis of these results indicates a high degree of statistical significance ($P < 0.001$). Ten osteosarcomas were positive for p16 and six were positve for EGFR, with only one tumor being positive for both. Two osteosarcomas were positive for p53, both of which showed concurrent EGFR staining.

Conclusions: p16, p53 and EGFR staining was limited to osteosarcoma, with the exception of one non-ossifying fibroma. The overall staining of these proteins is somewhat lower than rates reported in the literature for these lesions, which may be attributable to array sampling or more stringent criteria for positive staining. p16 and EGFR have complimentary patterns, as only one case showed staining for both. p53 did not independently identify osteosarcomas and was negative in all of the benign lesions. These patterns of immunoreactivity were able to differentiate osteosarcoma from benign bone tumors, and may be useful in diagnostically challenging cases.

34 Cluster Analysis of Diagnostic Immunohistochemical Markers in Leiomyosarcoma Delineates Specific Clinicopatholgical Subtypes

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Background: Leiomyosarcomas (LMS) represent a heterogenous group of tumors whose diagnosis is based on clinicopathologic findings that in general can be categorized into skin, soft tissue (ST), retroperitoneal/venous (R/V), visceral, bone and uterine subtypes. A number of markers for smooth muscle differentiation with differing sensitivities and specificities have been explored, however, limited information is known about the differential distribution of these markers by subtype. The purpose of this study is to analyze the differential expression of common smooth muscle markers, estrogen receptor (ER) and HMB-45 in a large series of LMS using tissue microarrays with cluster analysis.

Design: A total of 81 LMS were identified consisting of 51 non-uterine and 30 uterine tumors. The non-uterine LMS were subdivided into 28 skin/ST, 16 R/V, 4 visceral and 3 bone subtypes. Tissue microarrays were constructed, immunohistochemical staining intensity was scored as 0, 1+ or 2+, and cluster analysis was performed on the data.

Results: Among all tumors, SMA was the most sensitive (94%) followed by MSA (90%), calponin (88%), desmin (72%), and myosin and caldesmon (64% each). Myosin and caldesmon as a pair revealed parallel sensitivities within the various subtypes, and show the greatest sensitivity in the R/V group (94% each). ER positivity was identified almost exclusively in women with 63% of uterine LMS and 50% of R/V tumors (75% of positive tumors being from women). None of the non-uterine and only a single uterine LMS was positive for HMB-45. (See table).

Percentage of Positivity by Subtype

	Skin and ST (n=28)	Retro/Venous (n=16)	Visceral (n=4)	Bone (n=3)	Uterine (n=30)
Smooth Muscle Actin	96	100	100	100	87
Muscle Specific Actin	89	100	75	100	87
Calponin	86	100	75	100	83
Desmin	61	75	50	67	83
Myosin	50	94	50	67	63
Caldesmon	43	94	75	67	67
ER	11	50	0	0	63
HMB-45	0	0	0	0	3

Conclusions: Smooth muscle markers demonstrate variable sensitivities in LMS with the most sensitive marker being SMA. Myosin and caldesmon exhibit greatest sensitivity in R/V tumors. ER defines a distinct subgroups of LMS in the uterus and R/V neoplasms almost exclusively in women, which has important clinical implications. HMB-45 reactivity is rarely seen in LMS, unlike what others have recently reported.

35 Proteomic Evaluation of Osteosarcoma: Macrophage Migration Inhibitory Factor and Tumor Necrosis Factor-α Are Upregulated in a Highly Metastatic Murine Cell Line

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Background: Approximately one-third of patients with osteogenic sarcoma (OGS) die from lung metastasis within 5 years of diagnosis. Molecular signatures that predict pulmonary metastasis from primary OGS and identify those patients at highest risk would be clinically useful as prognostic markers.

Design: Protein expression profiles of two clonally related murine OGS cell lines with low (K12) and high metastatic (K7M2) potential were compared using two different proteomic technologies (two-dimensional difference gel electrophoresis (2D-DIGE) and tissue profiling by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS)) to detect candidate proteins that predict metastatic potential. Two such proteins were subsequently validated by Western blotting and immunohistochemical (IHC) analysis of a tissue microarray (TMA) containing 114 cases of primary human OGS, 56 of which either presented with or developed metastatic disease and 25 of which resulted in death.

Results: Comparison of the proteomic profiles of K12 and K7M2 cells revealed distinct differences in protein expression. Further molecular network analyses suggested several candidate molecules that potentially predict OGS metastasis to lung, including macrophage migration inhibitory factor (MIF) and tumor necrosis factor-α (TNFα). Western blotting confirmed increased expression of both molecules in lysates of K7M2 cells compared to K12 cells. IHC analysis of a human OGS TMA demonstrated that MIF and TNFα levels are increased in high grade OGS compared to low grade tumors (t-test, $p=0.0029$ and $p=0257$, respectively). However, log rank tests disclosed no significant differences in disease-free survival. IHC scores were not statistically different between patients who developed local recurrence or distant metastasis and those who did not. No significant changes in the levels of MIF or TNFα occurred after administration of adjuvant therapy or upon tumor progression.

Conclusions: Proteomic analysis of murine OGS cell lines disclosed several potential markers of pulmonary metastasis. Although upregulation of these candidate biomarkers for OGS metastasis were validated *in vitro*, IHC of a human OGS TMA did not confirm these findings. These results demonstrate the necessity of validation studies in the evaluation of molecular markers derived from animal studies using proteomic techniques.

36 Multicentric Osteosarcoma (MOS): A Clinicopathologic Review of 56 Cases

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Background: A very small number of osteosarcomas (OGS) present with multifocal skeletal lesions without visceral metastases. They are characterized as either synchronous (detected simultaneously) or metachronous (defined by a time interval between detection of the first lesion and subsequent lesions). There are only a few case reports or small series in the literature that address this topic. The purpose of this study was to examine a large series of patients affected by MOS in order to compare the clinicopathologic features of those with synchronous (S) to those with metachronous (M) tumors and assess factors that may help predict prognosis and guide treatment.

Design: Among a combined total of 3,832 patients with OGS who were treated at two large tertiary centers, we identified 56 patients with MOS without intervening visceral metastases. Histologic sections, radiographs and clinical information from the clinical charts were reviewed.

Results: There were 35 males and 21 females with a median age of 16 years (range 2 to 70 years). Six patients had an underlying genetic disorder predisposing them to OGS. 22 (39%) presented with synchronous OGS. 34 (61%) had metachronous tumors that developed in a median time of 22 months (mos) (range 2 to 171 mos). Histologically, all but one of the tumors were Grade 3 or 4 OGS. There were no differences in the clinical findings, site of involvement or histologic features between the S and M groups. The median survival in the M group was longer than the S group (median 43 mos vs 14 mos respectively; $P=0.001$) if calculated from the onset of disease in both groups. In contrast, there were no differences when comparing the M group median survival, calculated from the diagnosis of the second malignancy, and the S group, calculated from the beginning of disease (17 mos vs 14 mos respectively). These results were still valid when considering patients treated with (41) or without (15) chemotherapy as subgroups and when patients with genetic syndromes were excluded. However, 7 of the total 56 patients were considered cured and all of them were in the M group.

Conclusions: MOS is rare. There are no differences in the clinical findings, site of involvement or histologic features between patients who present with S or M tumor. While there are no differences in the median overall survival between the two groups, the only patients who were considered cured belonged to the M group. Therefore, aggressive individualized treatment in this group may result in cure.

37 Expression of Apoptosis-Related Genes in Sodium Selenite-Treated Osteosarcoma Cells

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Background: Selenium has long been considered as an essential trace element with several important biological functions. Recently, it has shown that selenium has anti-carcinogenic action and suggested that selenium-induced apoptosis underlies its cancer inhibitory activity. We explored the detailed changes in expression of the genes related with sodium selenite induced apoptosis in osteosarcoma cell line.

Design: Sodium selenite was added to cultured 3 osteosarcoma cell lines (SJSA-1, HS3.7 and MG63) with different concentrations (0.125, 0.25, 0.5, 1 and 2 mM) for 48 hours and with 0.25 mM for different time (3, 6, 12, 24, and 48 hours). Then the expression of Bcl-2, Fas, Apaf-1, p53, p21, Bax and p16 genes was analyzed using real-time RT-PCR.

Results: The Bcl-2 gene shows decreased expression according to the increased concentration of sodium selenite. The expression of p53, p21 and Bax genes was increased dose-independently. However, p53 expression was peaked in 3 hours after sodium selenite treatment, followed by p21 and Bax (peak concentration at 12 hours and 24 hours after treatment, respectively). The expression of Fas gene increased proportionally to the concentration and the exposure time of sodium selenite. The expression of p16 genes was also increased but dose- and time-independently. The Apaf-1 expression was variable according to the cell lines.

Conclusions: This study suggests that the sodium selenite would induce apoptosis in cultured osteosarcoma cell lines. And the possible mechanisms of apoptosis might be p53/p21/Bax pathway related to sodium selenite-induced DNA damage, Fas pathway, mitochondrial pathway related to the decreased Bcl-2 level, according to relevant changes in expression of apoptosis-related genes.

38 Expression of Insulin-Like Growth Factor-II mRNA-Binding Proteins 3 (IMP3) in Osteosarcoma

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Background: Insulin-like growth factor-II mRNA-binding proteins 3 (IMP3) is one of the RNA binding proteins implicated in the mRNA localization and translational control. It is expressed during embryogenesis, but also in some malignant tumors. In addition, recent studies suggest its potential as a prognostic factor or as a therapeutic target in malignancy.

Design: We examined the relationship between the clinical data including metastasis, survival and IMP3 immunohistochemistry staining in 47 osteosarcoma cases by using tissue micro array to evaluate whether IMP3 can be considered as a biomarker for any prognostic factors. From the 47 cases, 22 cases were of female patients and 25 cases were of male patients. The average age of the groups was 26 years old.

Results: In the tissue micro array containing 47 paraffin embedded osteosarcoma cases, 8 (17.02%) were proved positive for immunostaining of IMP3 and showed statistical correlation with tumor metastasis (p=0.02, Fisher's exact test). IMP3 expression was independent of survival, tumor site, age and sex.

Conclusions: These results support that IMP3 could be used as an independent prognostic factor in osteosarcoma with a high potential for metastasis.

39 EGFR and Its Downstream Molecules in Osteosarcoma

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Background: The correlations between the epidermal growth factor receptor (EGFR), its downstream molecules and their value as prognostic factor in tumors have been studied extensively. The aim of this study is to investigate whether these correlations exist in osteosarcoma.

Design: We evaluated the relationship between clinical data including metastasis and survival with EGFR and its downstream molecules, p-EGFR, AKT, STAT-3, survivin and p44 immunostaining in 47 cases of osteosarcomas. Of the 47 cases, 22 cases were of female patients, 25 cases were of male patients, and the average age of the groups was 26 years old. In addition, three cell lines of osteosarcoma were used for evaluation of EGFR expression level and mutation status by real time PCR and nucleotide sequence analysis.

Results: In tissue micro array containing 47 paraffin embedded osteosarcoma cases, 16, 20 and 12 cases showed positive immunostaing of STAT-3, survivin, and p44 respectively. STAT-3 expression didn't correlate with metastasis or survival. But survivin and p44 showed statistical correlation with survival (p=0.005 and p= 0.022, respectively, log rank test). Also a significant association between survivin expression and STAT-3 activation was observed (p=0.006, Fisher's exact test). EGFR staining was observed in two cases, and one of them showed metastasis. But it was hard to explain the correlation between EGFR and osteosarcoma statistically. p-EGFR and Akt immunostaining were not detected in any of the cases. Two of the three osteosarcoma cell lines showed increased EGFR in real time PCR. And one of them showed CAA to CAG mutation at ex20 of amplified EGFR gene, but this does not change the amino acid.

Conclusions: These results support the hypothesis that survivin and p44 are unfavorable prognostic factors in osteosarcoma. Further investigation will focus on the relationship between EGFR and ostosarcoma.

40 Coordinate Expression of Colony Stimulating Factor-1 (CSF1) and CSF1 Related Proteins Is Associated with Poor Prognosis in Leiomyosarcoma

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Background: The presence of increased numbers of macrophages is associated with poor prognosis in several malignancies. In a previous study we determined that CSF1 plays an important role in the tumorigenesis of tenosynovial giant cell tumor (TGCT), a tumor with large numbers of reactive macrophages (PNAS, 2006, 103:690-5). The aim of this is to evaluate the prognostic significance of coordinate expression of CSF1 and CSF1 related proteins in LMS.

Design: We performed in situ hybridization on a tissue microarray (TMA) containing samples from 149 cases of LMS to determine the RNA expression levels of CSF1 and the CSF1R. We performed immunohistochemistry on the LMS TMA to evaluate the expression of three proteins (CD163, CD16, CTSL), which are encoded by genes that are highly expressed in TGCT and which we hypothesize are associated with a coordinated response to CSF1 expression.

Results: In situ hybridization performed on the LMS TMA demonstrated that CSF1 expression was localized in the LMS cells, the majority of CD163 positive macrophages showed no expression of CSF1. In contrast, CSF1R was expressed in both tumor cells and macrophages. A subset of cases showed coordinate expression of CSF1 and three proteins (CD163, CD16, and CTSL) associated with the "CSF1 response" seen in TGCT. Kaplan-Meier survival analysis showed that tumors with coordinate expression of CSF1 and the three CSF1 related proteins showed significantly decreased disease specific survival in both non-gynecological LMS (Log Rank p=0.03) and gynecological LMS (Log Rank p<0.001).

Conclusions: This study demonstrates that CSF1 expression is localized within LMS tumor cells, and the coordinated expression of CSF1 with proteins associated with the "CSF1 response" is significantly associated with poor prognosis in LMS. The findings suggest that a coordinated "CSF1 response" may play an important role in the pathogenesis of LMS and that CSF1 and its receptor could serve as potential therapeutic targets.

41 Prognostic Factors in Synovial Sarcoma

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Background: Synovial sarcoma is an uncommon neoplasm showing dual epithelial and mesenchymal differentiation, accounting for 10% of all soft tissue sarcoma diagnoses. It has a characteristic translocation, t(X;18)(p11.2;q11.2) which represents a fusion product between the SYT gene on chromosome 18 and either SSX1, SSX2, or SSX4 on the X chromosome. However, additional genetic changes may be seen as the tumors advance. The purpose of this study is to analyze the available cytogenetic data on cases from within our department and additionally to perform reverse transcript polymerase chain reaction (RT-PCR) studies with the goal of correlating the data sets and identifying possible prognostic factors.

Design: Materials from 24 patients were collected and submitted for cytogenetic analysis, Most were performed by traditional karyotyping; only one patient had results analyzed by fluorescence in situ hybridization (FISH). Paraffin embedded archived materials were then analyzed using RT-PCR in 12 patients. The results were compared.

Results: 22 of 24 patients (92%) showed the characteristic t(X;18). 3 patients showed a three way translocation involving chromosomes X, 18, and a third chromosome. Nine of the 12 patients on which RT-PCR was performed showed fusion of the SYT gene with either SSX1 or SSX2. 3 of 12 patients (25%) showed co-existence of SYT-SSX1 and SYT-SSX2 in the same tumor. 3 of 24 patients had biphasic tumor and 2 of those patients expressed the SYT-SSX1 fusion product. All of the patients with the SYT-SSX2 fusion product had monophasic disease. Each of the patients with a three way translocation showed the SYT-SSX1 fusion product. Thirteen of the 22 patients (59%) with the characteristic translocation had additional chromosomal aberrations. 2 of 22 patients (9%) had an additional chromosome 19 and died of the disease. Two of 7 patients (29%) who showed the SYT-SSX1 transfusion transcript died of the disease. Two of 7 patients (29%) who showed the SYT-SSX2 transfusion transcript died of the disease. 1of 3 patients (33%) with a three way translocation also died of the disease.

Conclusions: Based on our data, additional chromosomal aberrations, namely changes in chromosome 19, may have more aggressive disease and a poorer prognosis. Three way translocations in conjunction with the expression of the SSX1 product may also have a poorer prognosis.

42 WT1 Expression in Benign, Borderline and Malignant Vascular Tumors: An Immunohistochemical Analysis of 67 Cases

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Background: Vascular tumors are commonly encountered in routine pathologic practice and often pose diagnostic challenges owing to their morphologic diversity. Recent studies suggested WT1 protein is an important regulator in endothelial proliferation. Although WT1 expression has been reported in some vascular tumors of skin, little is known about its staining patterns in a spectrum of vascular tumors from various locations, nor in lesions with non-neoplastic endothelial proliferation.

Design: Tissue microarray slides were prepared from representative tissue blocks of 67 cases of vascular tumors including angiosarcomas (AS, 19 cases), hemangioendotheliomas (HE, 5), Kaposi's sarcomas (KS, 4) cavernous hemangiomas (CVH, 12), capillary hemangiomas (CPH, 6), pyogenic granulomas (PG, 4), lymphangiomas (LA, 4), hemangiopericytomas (HP, 5) and glomus tumors (GT, 8). Four cases of granulation tissue were also included in the study for comparison. Routine hematoxylin and eosin and WT1 immunohistochemical stains were analyzed.

Results: Diffuse cytoplasmic positivity of WT1 was invariably observed in all cases of malignant vascular tumors including AS (19/19), KS (4/4) and HE (5/5). The staining intensity was unrelated to tumor locations, but stronger staining was often associated with spindle and solid morphology. Interestingly, WT1 was consistently expressed in some benign vascular tumors such as CPH (6/6) and PG (4/4), while it was exclusively negative in CVH (0/12) and LA (0/4). No WT1 staining was demonstrated in non-endothelial vascular tumors like GT (0/8) and HP (0/6), nor in non-neoplastic vascular proliferation of granulation tissue (0/4).

Conclusions: WT1 was invariably expressed in malignant endothelial cells in angiosarcomas, hemangioendotheliomas and Kaposi's sarcomas with a diffuse cytoplasmic pattern. This feature can be useful to distinguish malignant vascular tumors from poorly differentiated tumors mimicking a malignant vascular tumor. On the other hand, consistent WT1 staining in capillary hemangioma and pyogenic granuloma may help in differential diagnosis with non-neoplastic vascular proliferation or non-endothelial vascular tumors such as glomus tumor and hemangiopericytoma. The data suggest WT1 can be considered as a consistent and reliable endothelial marker in all malignant and some benign vascular tumors.

43 EWSR1-CREB1 Is Another Gene Fusion in Clear Cell Sarcoma of Soft Tissue

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Background: Clear cell sarcoma (CCS) is a distinct tumor of a melanocytic phenotype most commonly occurring in deep soft tissues. A recurrent chromosomal translocation t(12;22)(q13;q12) resulting in a fusion of the EWSR1 and the ATF1 genes is a cytogenetic

hallmark of CCS. Recently another fusion gene EWSR1-CREB1 has been identified in a unique subset of CCS that arose in the gastrointestinal tract and lacked melanocytic differentiation.
Design: In addition to standard clinicopathologic assessments, reverse transcription-polymerase chain reaction (RT-PCR) using RNA extracted from formalin-fixed, paraffin-embedded tissues of 33 CCSs arising in soft tissues was performed to detect the EWSR1-ATF1 and the EWSR1-CREB1 transcripts.
Results: The patients' ages ranged from 13 to 73 years, and there was a male predominance (20 males, 13 females). The tumors were located in the extremities (N = 25) or in the trunk or limb girdles (N = 8). The tumors were showed a nested fascicular pattern of spindle cells and/or a sheetlike growth pattern of epithelioid cells. Minor histologic variations were seen in 14 tumors, including rhabdoid cells (N = 8), bizarre pleomorphic cells (N = 6), alveolar structures (N = 3), and a seminomalike pattern (N = 2). Immunohistochemically, the tumors were positive for S-100 protein (33/33) and variably or focally for melanoma-associated markers such as HMB45 (32/33), MITF (26/32), and Melan A (23/32). In RT-PCR, variant transcripts (types 1-3) of the EWSR1-ATF1 were detected in 31 cases, and that of the EWSR1-CREB1 was found in the remaining two examples that retained melanocytic differentiation. There was no clear association between clinicopathologic findings and the detected fusion genes.
Conclusions: Our study demonstrats that CCSs of the soft tissue and gastrointestinal tract share identical genetic alterations despite somewhat divergent morphologic features, and suggests that molecular detection of the EWSR1-ATF1 and EWSR1-CREB1 is a valuable diagnostic molecular adjunct of CCS.

44 Pseudomyogenic ("Fibroma-Like") Variant of Epithelioid Sarcoma: A Distinctive Tumor Type with a Propensity for Multifocality in a Single Limb but Surprisingly Indolent Behavior

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Background: A 1992 report described 5 keratin-positive spindle cell neoplasms with multifocal presentation in a single limb, which were proposed to be a variant of epithelioid sarcoma (ES). This tumor type is not widely recognized and is incompletely characterized.
Design: We examined 29 cases of this distinctive tumor to evaluate histologic features. Immunohistochemistry was performed, and clinical follow-up was obtained.
Results: There was a 4:1 male predominance (median age 28 yrs; 86% ≤ 40 yrs). Half the pts presented with painful and half with painless nodules. Mean size was 1.8 cm (range 0.3-2.9 cm). Tumors involved lower limb (62%), upper limb (24%), or trunk (14%). Eighteen were multifocal (2-15 lesions), 11 in multiple tissue planes. In total, 19 involved dermis, 18 subcutis, 14 muscle, and 7 bone, all showing infiltrative margins. The tumors were composed of loose fascicles of plump spindle cells with vesicular nuclei, variably prominent nucleoli, and abundant brightly eosinophilic cytoplasm, some with a strikingly rhabdomyoblast-like appearance. In all cases, a minority of cells were epithelioid. Most tumors showed only mild atypia; 4 contained notably pleomorphic cells. Median mitotic rate was 1 per 10 HPF (range 1-10). Four tumors showed vascular invasion; 4 had necrosis. All were diffusely positive for AE1/AE3, 17/23 focal CAM5.2, 3/29 focal weak EMA, 9/26 focal SMA, and 1/28 PAN-K (MNF-116). All were negative for CD34, desmin, and S-100 and showed intact INI1. Follow-up ranged from 1-11 yrs (median 3.5 yrs). Most pts were treated by local excision. Ten had local recurrences or developed additional nodules in the same region, all within 10 mos. Four had post-operative XRT; 2 had chemotherapy. Three pts had amputations for multifocal disease. None have thus far developed distant metastases.
Conclusions: We describe a distinctive sarcoma type affecting mainly young men characterized by multifocality in different tissue planes of a limb. Although sharing some features with ES (skin/soft tissue of distal extremities; young adults; keratin positive), it differs by predominantly myoid-appearing spindle cell morphology, lack of EMA, CD34, and PAN-K expression, and intact INI1 (absent in 90% of conventional ES). Despite the ominous presentation, follow-up thus far suggests an indolent clinical course, at least in the first 5 yrs. Although its relationship with ES is uncertain, we propose the interim designation "pseudomyogenic" variant of ES.

45 NPY Receptors in Human Sarcomas: High Overexpression in Ewing Sarcoma Family of Tumors and Synovial Sarcomas

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Background: Many peptide hormone receptors are overexpressed in human cancer, allowing receptor-targeted scintigraphic tumor imaging and therapy with radiolabeled peptide analogs. A clinically established example is somatostatin receptor targeting of gut endocrine tumors. Neuropeptide Y (NPY) receptors are new candidates for these applications, based on their high overexpression in specific cancers like breast carcinomas. Since NPY receptors are known to be expressed in various sarcoma cell lines, the aim of the present study was to investigate in detail the NPY receptor expression in human sarcoma tissues.
Design: Eighty-one cases, including Ewing sarcoma family of tumors (ESFT), synovial sarcomas, desmoid tumors, lipo-, angio-, leiomyo-, rhabdomyo-, osteo- and chondrosarcomas, were investigated for their NPY receptor protein expression with *in vitro* receptor autoradiography using a ^{125}I-labeled universal NPY receptor ligand in competition with NPY receptor subtype-selective ligands. NPY receptor mRNA expression was assessed with in situ hybridization.
Results: In the autoradiography experiments, ESFT showed a remarkably high expression of the NPY receptor subtype Y1 on the tumor cells in terms of both receptor incidence (84%) and receptor density (mean 5314 dpm/mg tissue). Furthermore, synovial sarcomas were notable because they expressed Y1 in extremely high density on tumor cells (mean 7497 dpm/mg; incidence 40%). In liposarcomas and desmoid tumors, intratumoral blood vessels were frequently positive for Y1. The remaining sarcomas expressed NPY receptor subtypes Y1 and Y2 at low levels. In situ hybridization for Y1 receptor mRNA confirmed the autoradiography results of Y1 receptor protein expression.
Conclusions: NPY receptors are novel molecular markers for human sarcomas, with a particularly high expression in ESFT and synovial sarcomas. Biologically, Y1 may inhibit tumor growth, as previously shown in an in vivo mouse model of human ESFT. Regarding clinical applications, the high Y1 expression on tumor cells of ESFT and synovial sarcomas and on tumoral blood vessels of liposarcomas and desmoid tumors represents an attractive basis for an in vivo targeting analogous to somatostatin receptor targeting.

46 Reassessment of the Utility of Frozen Section Analysis for Hip and Knee Joint Revisions

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Background: Because of its reported high specificity and rapid turn-around time, intraoperative Frozen Section (FS) consultation has often been employed in evaluating the possibility of infection in cases of hip and knee prosthetic loosening. FS examination serves as an adjunct to pre-operative and intraoperative studies that include erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), aspiration with culture, radiological studies, intraoperative gram stain and culture, and surgical impression. Due to its apparent lack of optimal utility at our institution, we critically examined our experience in order to determine if FS examination had value as performed as a reflex test.
Design: For over an 11-year period, we retrospectively searched our surgical pathology database for every hip and knee surgical procedure that yielded a FS consultation. Any case in which the FS or permanent histologic section was interpreted as "positive" was reviewed microscopically. The criterion for the presence of acute inflammation, excluding surface exudate and fibrin, was the presence of greater than 5 polymorphonuclear leukocytes in a high-power field (hpf) in at least 5 separate hpfs. When available, cases from the database were searched for microbiologic culture results of surgical tissue specimens, the gold-standard, and compared with the FS findings.
Results: 277 cases, 149 with available culture results from surgical tissue samples, were identified. We found that only 21 cases (8%) demonstrated positive FS or permanent histologic section results. Fifteen (10%) of the 149 cases were positive for microorganisms on culture. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for FS examination were 53%, 94%, 50%, and 95%, respectively.
Conclusions: Used as a reflex test, FS analysis for assessing the presence of acute inflammation during joint revision surgery has reasonable specificity and negative predictive value. However, using FS examination as a reflex test results in poor sensitivity and positive predictive value. It is suggested that FS examination be used more selectively, such as for cases of suspected joint infection when other laboratory parameters, especially ESR and CRP, are elevated.

47 Molecular Genetic Evidence Supporting Idiopathic Retroperitoneal Fibrosis as a Clonal Proliferation

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Background: Idiopathic retroperitoneal fibrosis is a rare disease in which fibro-inflammatory tissue proliferates and typically surrounds the ureters, aorta, or iliac arteries. Idiopathic retroperitoneal fibrosis has been accepted as a disease since 1948 and its etiology remains unclear. We sought to determine if this lesion is a clonal process.
Design: Six cases of retroperitoneal fibrosis were identified from our surgical archives. Cases were selected based upon female sex, the absence of known causes of secondary fibrosis, and correlation with clinical symptoms of retroperitoneal fibrosis. The patients ranged in age from 19 to76 years (mean 45 years). Genomic DNA samples were prepared from formalin-fixed, paraffin-embedded tissue sections using laser-capture microdissection. X-chromosome inactivation analyses were performed on fibroblasts within the fibrous areas and compared to a control from each corresponding patient.
Results: All 6 cases were informative. Three (50%) showed non-random X-chromosome inactivation when compared to controls. In one of these three cases showing non-random X-chromosome inactivation, two separate specimens were submitted, with concordant X-chromosome inactivation pattern observed.
Conclusions: Our data suggest that idiopathic retroperitoneal fibrosis maybe associated with a clonal expansion of fibroblasts.

48 Gaucher Disease: Bone Histomorphology after Enzyme Replacement Therapy

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Background: Bone disorders in Gaucher disease are characterized variably by failure to remodel, osteopenia, lytic lesions and osteonecrosis, the latter often accompanied by pain and disability. Since 1994, enzyme replacement therapy (ERT) has provided clinical improvement by pain relief, reduction in bone crises and fatigue, evident 12-20 weeks after treatment. Clinically, orthopedic surgeons have found, that compared to patients with untreated disease, bone in Gaucher disease patients with ERT has normal material properties. To understand bone effects of ERT in Gaucher disease, we examined the bone histology after 1-9 years of ERT therapy.
Design: Femoral heads were obtained from total hip arthroplasty in four female patients (age 24-64 years) who had Gaucher disease diagnosed in childhood and who had ERT for 1-9 years prior to hip replacement. Bone blocks including a mid coronal block were x-rayed, fixed in formalin, decalcified, embedded in paraplast and 5-µm H&E sections prepared. These were compared to marrow biopsies obtained pre-therapy at the time

of diagnosis. In one case, undecalcified bone blocks, embedded in Spurr resin, were prepared and 5-µm sections were cut and stained with von Kossa and H&E stains to assess bone mineralization.
Results: In contrast to pretreatment bone marrow biopsies where Gaucher cells are surrounded by haematopoietic marrow, after one year ERT, the marrow showed some adipose tissue associated with groups of Gaucher cells and areas of haematopoietic tissue. By nine years of ERT, relatively few Gaucher cells were observed in the predominately adipose tissue bone marrow. The bone showed focal osteonecrosis. However, the surrounding bone trabeculae were lined by osteoblasts and contained viable osteocytes. New bone formation was seen at the interface of the osteonecrosis and the viable bone. The undecalcified sections showed fully mineralized bone.
Conclusions: Although morphologic improvement was seen as early as one year, ERT failed to prevent osteonecrosis. This suggests that onset of osteonecrosis preceded therapy. ERT did revert bone marrow elements towards the normal mixed hematopoietic adipose tissue pattern and restored bone tissue beyond the osteonecrotic areas towards cellular architecture typical of normal trabecular bone. Therefore, ERT can fully reverse both the hematopoietic and bone manifestations of Gaucher disease.

49 KIT codon 558 Insertions Are Rare Mutations Preferentially Occuring in Intestinal GISTs and Migth Indicate Increased Risk of Malignant Behavior in Gastric Tumors

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Background: Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of gastrointestinal (GI) tract. A majority of GISTs is driven by oncogenic mutations in KIT juxtamembrane domain (exon 11). These mutations consist of deletions, deletion-insertions, substitutions, duplications, and insertions (ins). The latter occur exclusively in codon 558. A clinicopathologic profile of GISTs with ins is unknown.
Design: 16 GISTs with ins in 5' KIT exon 11 were selected from > 1500 KIT mutants collected by collaborating institutions.
Results: 14 of 16 (87.5%) mutations consisted of 3 nucleotide ins (1694_1695insTCC) leading to K558delinsNP. However, 2 variant ins (1694_1695insCAA and 1694_1695insCAAC, the latter coexisting with deletion of 1695delG) leading to K558delinsNQ and K558delinsNN were identified, respectively. Median age of the patients was 60 years. Male to female ratio was 1:1.6. GISTs with ins were diagnosed in stomach (n=4), small intestine (n=7), and rectum (n=2). 3 tumors were disseminated and the primary location could not be established. 13 tumors had spindle cell morphology and epithelioid cell features were seen in only 3 intestinal GISTs. Based on the size and mitotic activity, and clinical follow up data, 10 of 16 (62.5%) GISTs were diagnosed as malignant. 2 of 4 (50%) gastric and 5 of 9 (55.6%) intestinal GISTs had >50% risk of metastases. 2 gastric and 4 intestinal GISTs were classified as benign with low to moderate risk of metastases. Previously, clinicopathologic data have been reported in 3 of 8 GISTs with K558delinsNP; there were 2 malignant gastric GISTs and 1 malignant small intestinal tumor.
Conclusions: KIT codon 558 ins are rare and account for <1% of KIT mutants. GISTs with such mutations are more common in females. Although KIT ins can occur in tumors from different parts of the GI tract, they are 2 times more frequent among intestinal GISTs. Based on this study and a review of the literature, presence of 5' KIT exon 11 ins might indicate an increased risk for malignant behavior among gastric GISTs. However, more cases should be studied to confirm this observation.

50 Rhabdomyoblastic Differentiation in Gastrointestinal Stromal Tumors after Tyrosine Kinase Inhibitor Therapy

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Background: Approximately 80% of advanced gastrointestinal stromal tumors (GIST) show responses to the tyrosine kinase inhibitor (TKI) imatinib mesylate, but most pts develop resistance after a median of 2 yrs. In general, progressing GISTs retain typical morphology. Herein we report 4 cases of metastatic GIST with heterologous rhabdomyoblastic differentiation after treatment with TKI.
Design: Immunohistochemical and mutational analyses were performed on histologically classic GIST and on components with rhabdomyoblastic differentiation.
Results: There were 2 males and 2 females (aged 35-66 yrs). 3 tumors were localized (2 stomach; 1 small bowel), and one disseminated (peritoneum, liver, pancreas). The disseminated GIST showed spindle cell morphology and was CD117-negative, whereas the others were CD117-positive and epithelioid, including 1 with marked pleomorphism. All localized tumors were high risk, and following resection developed intra-abdominal (2 pts) and liver (1 pt) metastases. All pts were treated with imatinib and showed partial responses (3 pt) or stable disease (1 pt), but subsequently progressed; 1 pt was then treated with sunitinib with minor response. 3 pts underwent surgical debulking. Follow-up from presentation was 20-87 mos: 2 pts died of disease (1 with metastases to lung, vertebra, and paraaortic LNs), and 2 were alive with disease. Rhabdomyoblastic differentiation was present in all pts in at least one metastatic site, adjacent to areas of classic GIST. The rhabdomyoblastic component resembled pleomorphic (3 pts) or embryonal rhabdomyosarcoma (1 pt) with strong, diffuse positivity for desmin and myf-4, whereas CD117 was negative. Mutational analysis performed on the post-TKI specimens revealed the same RTK mutations in both the conventional GIST and rhabdomyoblastic components: *KIT* exon 11 mutations in 3 cases (heterozygous V559D in 2; homozygous deletion 556-574 in 1), and a *PDGFRA* exon 18 deletion in 1 case.
Conclusions: We report for the first time rhabdomyoblastic differentiation arising in GISTs that progressed on TKI therapy. The heterologous component showed typical morphologic/immunophenotypic features of rhabdomyosarcoma and retained the RTK mutation of the precursor GIST. This unusual finding (with loss of CD117 expression) can represent a diagnostic pitfall. The molecular mechanisms for this form of TKI-resistant clonal evolution remain to be determined.

51 Morphologic and Molecular Heterogeneity in Imatinib-Resistant GIST

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Background: Many GIST patients (pt) develop clinical resistance to the KIT/PDGFRA kinase inhibitor imatinib (IM). However, it is unclear whether clinical resistance results from single or multiple molecular mechanisms in individual patients.
Design: 57 GIST samples from 14 pt undergoing debulking after clinical progression on IM were screened for *KIT* and *PDGFRA* mutations (2-to-7 samples/pt). Samples were selected for differences in anatomic location, morphological appearance (spindle, epithelioid, mixed or unusual), or CD117 expression. Mutations were identified using complementary techniques, including denaturing high-performance liquid chromatography (KIT exons 9, 11 and 12-18), allelic cDNA sequencing, and mutation-specific PCR (V654A, D820G, N822K, Y823D), which have sensitivities, respectively, of 10%, 5% and 0.5-5%.
Results: Primary *KIT* oncogenic mutations were found in 11 of 14 pt (79%). Nine of these 11 pt (83%), had secondary drug-resistance mutations in the *KIT* kinase domain. Six pt (67%) had 2 or more secondary mutations among different samples, and 3 pt (34%) had 2 or more secondary *KIT* mutations within individual metastases. The secondary mutations clustered in the KIT ATP binding pocket (~39%) and kinase catalytic region (~61%). Notably, allelic cDNA sequencing studies showed multiple novel *KIT* kinase domain mutations, expressed at low levels, beyond the predominant secondary mutations summarized above. Unusual morphologic features in 4 pt included pleomorphic spindle cells (1 pt WT) and huge epithelioid cells with eosinophilic cytoplasm and intracytoplasmic inclusions [3 pt, one each exon 11, exon 13 and WT]. In 4 cases, including 2 with bizarre histologic features, there was loss of CD117 expression. No secondary resistance mutations were identified in the specimens showing bizarre histology or loss of CD117 expression.
Conclusions: IM resistance is commonly due to the selection/acquisition of secondary mutations within the kinase domain. Our findings reveal that in many pt there is striking inter- and intra-lesional heterogeneity in these mutations. In addition, some tumors appear to dedifferentiate and lose CD117 expression. These observations underscore the problem with kinase inhibitor monotherapy and raise concern over the ultimate effectiveness of second- and third-line inhibitors in IM-resistant pts.

52 Early Angiogenesis in the Pathogenesis of Human Sarcomas. An Immunohistochemical, Ultrastructural and Molecular Study

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Background: Animal models are widely used in the study of vascular neogenesis of tumors. We have used a nude mice model to study the earlier stages of this process in xenotransplanted in human sarcomas. Aim of this study is to characterize at histological, immunohistochemical, ultrastructural and molecular level the neovascularization established between the xenotransplanted tumor and the host.
Design: Five human sarcomas were evaluated: a Ewing's sarcoma (ES), an osteosarcoma (OS), a chondrosarcoma (Chs), a synovial sarcoma (SS), and a gastrointestinal stromal tumor (GIST). Tumor pieces of 0.3-0.4 cm in size were implanted in the back of a series of nude mice. The animals were sacrificed at 24, 48, 72 hours, 7, 14, 21 and 28 days from implantation. The histological and immunohistochemical studies with several angiogenic factors (VEGF, KDR, Flt1, Flt4, VE-CAD, PDGFRA, HIF-1±) were complemented with electron microscopy. RNA was obtained from fresh tissue and the expression of 96 angiogenesis related genes was evaluated by means of quantitative RT-PCR using the micro-fluidic card technology.
Results: After 24-48 hours from tumor implantation, in all cases there is an intense expression of several angiogenic factors. Simultaneously the stroma surrounding the graft initiates angiogenesis. Early new vessel sprouting from the periphery infiltrates the tumor as isolated endothelial cells which mix together with tumor cells producing pseudo- vascular figures (vascular mimicry), which are progressively occupied with erythrocytes. Interestingly, cluster analysis of the angiogenic factors expression showed that the expression profiles at 48h and 1 week had the same behavior and in turn these correlated with the angiogenesis induction and the angiogenic remodeling within the tumor respectively. In addition, two different groups of tumors were observed according to the expression profiles, one constituted by the Chs and ES and the other by the GIST, OS and SS
Conclusions: Both induction and remodeling stages can be distinguished in the early angiogenesis process of human sarcomas. These models could constitute a useful tool in the study of tumor progression as well as in the evaluation of new anti-angiogenic drugs. This study has been partially funded by grants PI040822 from the Instituto Carlos III de Madrid, Spain and Contract nº: 018814 (EuroBoNet) from the 6thFP of the EC.

53 Can Lymphangiosarcoma Be Resurrected? A Clinicopathologic and Immunohistochemical Study of 51 Cases

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Background: In the past, lymphangiosarcoma and hemangiosarcoma were considered distinct entities separated mainly by clinical characteristics. Currently, all malignant endothelial tumors are called angiosarcoma. A number of morphologic features, including kaposiform architecture and hobnail endothelial cells, are thought to be associated with lymphatic differentiation in benign and malignant vascular tumors. Recently a number of immunohistochemical markers specific for lymphatic endothelial differentiation have been discovered. In order to see if there exists a subset of angiosarcoma with lymphatic differentiation, we analyzed clinicopathologic and immunohistochemical features in a series of 51 cases.

Design: Tumors were divided into 4 clinical groups: sporadic cutaneous, visceral/deep-seated, radiation-associated or lymphedema-associated. Architecture was categorized as kaposiform, vasoformative or solid, and cytology as hobnail, epithelioid, spindled or pleomorphic. Tissue microarrays were constructed and immunostained.

Results: There were 23 sporadic cutaneous, 15 visceral/deep-seated, 9 radiation-associated and 4 lymphedema-associated tumors. 26 tumors had kaposiform architecture and/or hobnail cytology. Among the kaposiform and/or hobnail tumors, D2-40, prox-1 and LYVE-1 were positive in 68, 52 and 38%, respectively, whereas in the other tumors they were positive in 47, 41 and 47%, respectively. In addition, the kaposiform and/or hobnail tumors were more likely to be well differentiated, 23 vs. 8%. Among 8 tumors positive for all 3 of these lymphatic markers, 88% had hobnail morphology. Many tumors with immunomorphologic evidence of lymphatic differentiation had additional solid areas with epithelioid cytology consistent with areas of tumor progression/dedifferentiation. There was no correlation between clinical category and morphologic or immunohistochemical evidence of lymphatic differentiation. CD31, CD34 and thrombomodulin were positive in 81, 58 and 62% of all tumors, respectively, and were positive in similar numbers of tumors with or without evidence of lymphatic differentiation.

Conclusions: Based on the association between kaposiform and/or hobnail morphology and staining for D2-40 and prox-1, there appears to be a subset of angiosarcoma that might be considered lymphangiosarcoma. These tumors are more likely to be well differentiated, but do not have distinct clinical features. D2-40 and prox-1 may be useful for supporting a diagnosis of lymphangiosarcoma.

54 Diagnostic Utility of a Newly Designed Fusion-Type-Specific RT-PCR and FISH in Clear Cell Sarcoma

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Background: Clear cell sarcoma (CCS) is a rare malignancy of young adults with predilection for the lower extremities and high propensity for regional or distant metastases. Because CCS and melanoma share histological, immunophenotypic and ultrastructural features, differentiating these two entities can be difficult. Most CCSs carry a recurrent t(12;22)(q13;q12) that may result in four reported *EWSR1/ATF1* fusion types. To date, few studies have attempted to characterize these four fusion types. Therefore, it is desirable to develop a fusion-type-specific molecular assay to fully characterize this tumor, particularly for formalin fixed, paraffin embedded (FFPE) tissue samples.

Design: Twenty-seven FFPE specimens, 25 CCSs and 2 melanomas (used as negative controls) collected between 1988 and 2007, were tested in blinded fashion using a novel reverse transcription polymerase chain reaction (RT-PCR) assay that detects and distinguishes the 4 reported *EWSR1/ATF1* CCS fusion types. The assay was optimized for FFPE archival samples, generating amplicons between 130-175bp, that were confirmed by direct DNA sequencing. Fluorescence in situ hybridization (FISH) using an *EWSR1* break-apart probe was concurrently performed in all cases available (22/25).

Results: (1) 17/25 CCSs had RNA of sufficient quality for RT-PCR testing. (2) RT-PCR detected *EWSR1/ATF1* fusion transcripts in 14/17 cases. 13 were type 1 (*EWSR1* exon 8/*ATF1* exon 4), among which 6 tumors also carried type 2 fusions (*EWSR1* exon 7/*ATF1* exon 5). One case carried both type 2 and type 3 fusions (*EWSR1* exon 10/*ATF1* exon 5). (3) FISH was performed on 22 CCS cases, and 17/22 were positive for *EWSR1* rearrangement. (4) In all CCS cases with sufficient RNA quality, results from RT-PCR and FISH were 100% concordant. (5) Both melanomas were negative for rearrangements by both molecular tests.

Conclusions: (1) We successfully developed a sensitive and reproducible RT-PCR assay to detect and distinguish all reported *EWSR1/ATF1* fusion types in CCSs. (2) The type 1 fusion was the most prevalent in our study. (3) More than one fusion type was detected in 7/14 positive cases. (4) Although *EWSR1* FISH cannot differentiate among different fusion types, we found it efficacious, especially when RNA quality is suboptimal for RT-PCR.

55 Frequency of *HMGA1* Rearrangements in Spindle Cell and Pleomorphic Lipomas

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Background: Spindle cell and pleomorphic lipomas are benign subcutaneous adipose tissue neoplasms that typically occur in the neck and upper trunk of older men. Conventional cytogenetic analysis often shows monosomy or partial losses of chromosomes 13 and 16, but rearrangements of 6p21 have been occasionally reported in these tumors. While one study has identified *HMGA1* overexpression by immunohistochemistry in one case of spindle cell lipoma (Dumollard JM et al Ann Pathol 2001), another demonstrated *HMGA1* rearrangement by fluorescence in situ hybridization (FISH) in a distinct case (F Tallini G et al Lab Invest 2000, 80:359-369). Because we have recently encountered a classic case of spindle cell lipoma in the face of a 66-year-old man with rearrangement of chromosome 6p21, we decided to investigate the frequency of *HMGA1* rearrangements in a series spindle cell and pleomorphic lipomas.

Design: Seventeen spindle cell lipomas and four pleomorphic lipomas were studied for rearrangements of *HMGA1* and *HMGA2* by FISH on paraffin-embedded thin sections. Fresh and frozen tissues were available from the index case for standard cytogenetic analysis and semi-quantitative reverse-transcriptase polymerase chain reaction (RT-PCR). Immunohistochemistry for CD34 and S100 was performed in all cases.

Results: Cytogenetic analysis was performed in one spindle cell lipoma and demonstrated the following karyotype: 46,XY,t(1;6)(p32;p21.3),del(13)(q12q14)[19]/46,XY[1]. FISH analysis confirmed rearrangement of *HMGA1*, and semi-quantitative RT-PCR showed transcriptional upregulation of this gene. Molecular cytogenetic studies of 16 additional spindle cell lipomas revealed rearrangement of *HMGA1* in only a single spindle cell lipoma. *HMGA1* rearrangements were not encountered in pleomorphic lipomas. *HMGA2* rearrangements were not found in any of the cases studied. All cases were positive for CD34 and negative for S100 by immunohistochemistry.

Conclusions: Rearrangements of chromosome 6p21 with involvement of *HMGA1* seem to occur in approximately 10% of spindle cell lipomas (2/17 in the present series), a frequency similar to that observed in ordinary lipomas. This finding suggests that *HMGA1* may play a role in the pathogenesis of a subset of these tumors. Since the number of pleomorphic lipomas studied in this series was small, we cannot rule out the possibility that *HMGA1* rearrangements may also occur in some of these tumors.

56 Intra-Articular Synovial Sarcoma (IASS): A Study of 9 Cases

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Background: Intra-articular synovial sarcoma (IASS) is extremely rare, with only scattered case reports in the literature. The general impression is that these lesions are frequently small and relatively indolent. IASS may simulate benign conditions, thereby leading to intralesional excision and potential joint contamination. The histological features of IASS may be deceptively bland, leading to the misdiagnosis of a benign lesion. RT-PCR to detect SYT/SSX fusion transcripts in such cases would therefore improve diagnostic accuracy. At this point in time, the optimal treatment of IASS is not clearly defined.

Design: We retrospectively reviewed the clinical and histological features of 9 cases of intra-articular synovial sarcoma and performed RT-PCR.

Results: There were 5 males and 4 females, 15 - 37 years of age (median 20 years). Seven cases occurred in the knee joint, 1 in the hip and 1 in the finger. Tumor sizes ranged from 8 mm to 7.5 cm (hip). The most frequent clinical impressions were pigmented villonodular synovitis and synovial chondromatosis. Three patients had longstanding joint pain (2, 5 and 10 years) before diagnosis. Histologically and radiologically, many of the cases were heavily calcified. The majority of cases were classified as monophasic synovial sarcoma; however, one was biphasic and 2 were poorly differentiated. The 2 poorly differentiated cases were associated with lung metastases 6 and 10 months after diagnosis; one of these also had lymph node metastases. None of the other cases had early metastases. RT-PCR detected SYT/SSX fusion transcripts in all 4 studied cases. The majority of patients were treated with limb salvaging surgery; 2 underwent amputation. The two patients with poorly differentiated tumors received chemotherapy pre-operatively followed by resection or amputation.

Conclusions: 1) The clinical behavior of IASS is strongly influenced by the presence of poorly differentiated areas. 2) Conservative resection of IASS without poorly differentiated areas is probably warranted, provided there is close longterm follow-up. 3) Radical surgery and chemotherapy do not appear to prevent metastases in poorly differentiated intra-articular IASS. 4) RT-PCR is a useful adjunct in confirming the diagnosis of cytologically bland monophasic IASS.

57 Insulin-Like Growth Factor (IGF)-I and IGF-I Receptor (IGF-IR) Are Consistently Expressed in the Most of Chordomas

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Background: Insulin-like growth factor (IGF) system has been implicated in tumor development and progression in various neoplasms including osteogenic and soft tissue sarcomas. We have been interested in the relationship between chordomas and IGF system, since among 183 bone and soft tissue tumors, only chordomas and liposarcomas showed consistent expression of IGF-I [Mod Pathol 2006;19 and Mod Pathol 2007;20]. Chordoma is a unique and low to intermediate grade malignant tumor that recapitulates notochord; however, little is known about the etiologic factors that predispose to them. The expression of IGF-I appeared to be implicated in the growth of chordomas according to our previous study, and we increased the number of cases to further investigate the relationship between IGF system and chordomas.

Design: Formalin-fixed, paraffin-embedded tissues from 14 patients (mean age 67.2 years, M:F=8:6) with chordomas who underwent surgical resection were used in this study. Two patients had 3 recurrences, and one patient had 1 recurrence (21 specimens in total). Histologically, all tumors are characterized by lobules and fibrous septa, and consisted of vacuolated round to oval cells with physaliphorous cells proliferated in the myxoid stroma. Immunohistochemical studies for IGF-I, IGF-I receptor (R) on these tumors were performed with or without antigen retrieval. Appropriate positive and negative controls were run together with the cases.

Results: Nine of 14 cases were strongly positive for IGF-I (cytoplasmic), weakly positive in 3 cases, and negative in 2 cases. IGF-IR (cytoplasmic and membranous) was strongly expressed in 12 of 14 cases, and weakly expressed 2 cases. The expression levels of IGF-I and IGF-IR were consistent in the 3 cases with recurrences. The spindle

cell (sarcomatous) component in the one case showed diminished expression of IGF-I, in spite of a good expression in the typical chordoma area.
Conclusions: These data strongly support the involvement of IGF-I and IGF-IR in the growth of the most part of chordomas. In addition, the expressions of IGF-I and IGF-IR in the recurrent tumors were consistent in the same patient. The diminished expression of IGF-I in the sarcomatous component may explain the diversity of tumor growth in the chordoma. The treatment of chordomas is difficult and wide surgical excision is desirable, but rarely feasible based on the anatomic location of the tumor. This study may offer new targets for therapeutic intervention in the management of inoperable and recurrent chordomas.

58 Detection of CHOP Gene Break–Apart by Fluorescence In Situ Hybridization (FISH) in Formalin Fixed Paraffin Embedded Myxoid/Round Cell Liposarcoma

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Background: Myxoid/round cell liposarcoma (MRCL), the most common subtype of liposarcoma, is frequently difficult to distinguish from other myxoid mesenchymal neoplasms, such as myxoid lipoma and myxoid sarcoma, with conventional pathologic approaches, especially, on small biopsy tissue. Chromosomal translocations of t(12;16)(q13;p11) and t(12;22)(q13;q12), rendering gene fusions of FUS (TLS)-CHOP or EWS-CHOP, have been found to be characteristic for MRCL and can potentially be used as molecular markers for the diagnosis of MRCL.
Design: Commercial FISH probe for CHOP gene break apart, which can detect both translocations involving CHOP gene, was used to test 10 cases of myxoid liposarcoma and 10 other mesenchymal neoplasms (1 pleomorphic sarcoma, 1 rhabdomyosarcoma, 2 osteosarcoma, 1 PNET, 2 Ewing's sarcoma, 1 myxoid lipoma, 2 myxoid sarcoma). To demonstrate accuracy of the assay, FISH on metaphase of human normal cells and RT-PCR were also performed.
Results: All the cases tested showed bright FISH signals with clear background. The FISH probe was specifically localized at 12q13 regions on metaphase of normal cells without cross-hybridization with any other chromosome. Ten (10) cases of myxoid liposarcoma were all positive for CHOP gene break-apart (100% test sensitivity). However, none of the ten (10) other mesenchymal neoplasms were positive (100% test specificity). The FISH results were confirmed by our RT-PCR assay with 100% consistency.
Conclusions: FISH for CHOP gene break-apart on paraffin-embedded tissue is a sensitive and specific assay in the detection of chromosomal translocations involved in myxoid liposarcoma, and can be used as a useful adjunct to the disease diagnosis and differential diagnosis.

59 New Aspects in the Cytogenetic and Ultrastructural Analysis of Chondromyxoid Fibroma

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Background: Chondromyxoid fibroma is a very rare neoplasm accounting for less that 1% of all bone tumors. To date, there are only 12 published cases with abnormal karyotype. Most of these cases describe involvement of the long arm of chromosome 6. Ultrastructural studies of this tumor have been done mostly in the past. Electron microscopy typically reveals numerous microvilli-like irregular cell processes, pinocytotic vesicles and invaginations of the cell membrane.
Design: Eight cases of chondromyxoid fibroma (CMF) were included in this study that aimed at identifying ultrastructural and chromosomal aberrations. The results were compared to the previously reported data. There were four women and four men included in the study. Age ranged from 41-55 years of age. Locations included tibia, ilium, ulna, radius, index finger and clivus. Fresh tissue of the tumors was submitted for cytogenetic analysis. Additionally, formalin-fixed tissue was submitted for light microscopy, and gluteraldehyde-fixed tissue was submitted for electron microscopy.
Results: Four out of eight cases had a cell culture producing metaphases amenable to karyotyping. All cases showed chromosome 6 abnormality in the 13q region. One case had a pericentromeric inversion of chromosome 6; two cases had deletion of the long arm of the chromosome 6 at the 13q region; and the fourth case had t(6;10)(q11.2;q13) translocation, not previously reported in the literature. Ultrastructural studies were performed and revealed interesting findings. There were numerous abnormal centrioles present in one of the cases which had nine triplets of microtubules assembled in a circle with triplets containing two normal and one abnormal, very small microtubule.
Conclusions: The abnormally-formed centrioles may correlate with unusual cellular and nuclear morphology of CMF. Comparison of our results with previously reported cytogenetic studies confirms the diagnostic utility of identifying 6q13 rearrangement. In contrast to some of the previous studies, we conclude that the sole 6q13 rearrangement is the marker of CMF, regardless of additional findings of pericentromeric inversion of chromosome 6 or translocations involving other chromosomes. Various translocations found in CMF are representative of the spectrum of CMF karyotype with a constant feature of 6q13 rearrangement. Review of the previously described karyotype abnormalities revealed that in those cases, where a different locus of 6q was reported, it was very close to the q13 region.

60 Integrin-Linked Kinase (ILK) and Its Binding Partners Are Implicated in the Pathogenesis and Progression of Human Chondrosarcomas

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Background: Chondrosarcomas (CHS) are the second most common primary skeletal malignancies. However, the molecular events underlying their pathogenesis are still obscure. Integrin-linked kinase (ILK) is an important component of cell–matrix adhesions implicated in vital processes of many cells, including chondroblasts. Furthermore, it has been associated with anchorage-independent growth, cell scatter, and carcinogenesis. ILK binds to the focal adhesion proteins α-parvin, β-parvin and PINCH, forming a stable adhesion complex. Mitogen-inducible gene-2 (Mig2), kindlin, and migfilin are recently identified adhesion molecules. Recent hepatocyte-culture studies have shown that the ILK–PINCH–parvin complex interacts with Mig2. The purpose of the present study was to investigate the roles of the ILK–parvin complex and of Mig2, migfilin, kindlin in cartilaginous tumors pathogenesis and to assess these molecules as possible markers for the prediction of CHS clinical behavior.
Design: Our material included sections from 60 paraffin-embedded well-characterized CHS of all grades and 25 enchondromas (ECH). We performed immunohistochemistry using mouse monoclonal antibodies that we generated against ILK, α-parvin, β-parvin, Mig-2, migfilin, and kindlin.
Results: Positive nuclear and cytoplasmic immunostaining for ILK, α-parvin, β-parvin, and Mig2 was observed in 56/60, 52/60, 57/60, 50/60 CHS and in 8/25, 4/25, 18/25, 10/25 ECH, respectively; migfilin and kindlin were not detected under the conditions employed. The percentage of cell positivity for ILK, α-parvin, β-parvin, and Mig2 were significantly higher in CHS compared to ECH ($p<0.001$ for all). The cellular levels of ILK and Mig2 were significantly augmented in high-grade (HG) (G2/3) compared to low-grade (LG) (G1) CHS and in LG tumors compared to ECH ($p<0.005$ for all). Normal chondrocytes were not immunoreactive. The expression levels of ILK/α-parvin/Mig2 were significantly correlated to each other (Kendal's $t = 0.44$-0.809, $p<0.01$). Finally, ILK predicted accurately HG CHS (overall accuracy 82%).
Conclusions: 1) The ILK–α-parvin–Mig2 adhesion complex is expressed in chondrogenic tumors; 2) ILK and Mig2 maybe associated with CHS development; 3) ILK can be used as a molecular marker to identify grade-based aggressive CHS; 4) highly-selective agents targeting ILK might constitute novel complementary therapy for CHS patients.

61 Expression of Integrin-Linked Kinase (ILK) and Its Binding Partners in Human Leiomyosarcomas

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Background: Leiomyosarcomas (LMS) are malignant neoplasms composed of cells that exhibit distinct smooth muscle differentiation. The molecular and cytogenetic features of LMS are complex. Integrin-linked kinase (ILK) is an important component of cell-matrix adhesions, implicated in vital cell processes, namely embryonal development, differentiation and survival. Furthermore, ILK overexpression has been associated with anchorage independent growth, cell scatter and ultimately carcinogenesis. ILK is binding to the focal-adhesion proteins α-parvin, β-parvin and PINCH, forming a stable adhesion complex. The aim of this study was to determine the expression and distribution of ILK and its binding companions α-parvin and β-parvin in human soft tissue LMS and to investigate their implication in LMS pathogenesis.
Design: We generated mouse monoclonal anti-ILK, anti-α-parvin and anti-β-parvin antibodies and performed immunohistochemistry on a human tissue microarray (TMA) composed of 40 LMS (25 primary, 6 locally recurrent and 9 metastatic) of all grades.
Results: Normal smooth muscle cells did not stain for any of the examined proteins, under the conditions employed. However, increased nuclear and cytoplasmic immunoreactivity for ILK, α-parvin and β-parvin was observed in the 39/40, 32/40 and 38/40 of LMS, respectively. The expression levels of ILK and β-parvin were significantly correlated to each other (Kendall's Tau test, p=0.032). A-parvin displayed considerably increased intensity of staining reaction in recurrent/metastatic compared to primary LMS (Mann Whitney U test, p=0.02).
Conclusions: 1) ILK and its binding partners are widely expressed in human LMS and may have a role in their pathogenesis and progression. 2) It is possible that ILK/β-parvin serves as one of the principal focal adhesion complexes in LMS pathobiology.

62 Involvement of the p38/MAPK – NF-κB Signal Transduction Pathway and COX2 in the Pathobiology of Meniscus Degeneration

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Background: Meniscal tears are attributed either to trauma or to degeneration processes. *In vivo* and *in vitro* data suggest that the morphological and biochemical alterations of fibrocartilage during meniscal degeneration (MD) are associated with knee osteoarthritis (OA). However, the molecular events underpinning human MD remain elusive. Nuclear factor-κB (NF-κB) is a homo-/hetero-dimeric transcription regulator activated by numerous kinases, including p38/MAPK. The most abundant NF-κB complex is the p50–p65 heterodimer. In its cytoplasmic (inactive) form, NF-κB dimer is bound to I-κB family of inhibitory proteins. Upon phosphorylation, NF-κB dissociates from I-κB and translocates to the nucleus, promoting, among others, the transcription of *cox2*, a gene engaged in inflammatory processes. The aim of the present study was to determine the immunoexpression of p38, its phosphorylated (activated) form p-p38, its substrate NF-κB (p50–p65), and COX2 in raptured menisci chondrocytes and to explore their involvement in the pathobiology of MD and related OA.
Design: Seventy menisci were obtained from patients with (n=20) and without (n=50) concurrent OA. Antibodies against p38, p-p38, NF-κB/p50, NF-κB/p65, and COX2 were employed for immunohistochemistry. The expression levels of these proteins were correlated to the presence of MD and to clinical parameters (symptom duration and OA).
Results: 1) MD was observed in 52% of the menisci; 2) p38 and COX2 displayed almost exclusively cytoplasmic, whereas p-p38 primarily nuclear localization. NF-κB species were detected in both the nucleus and the cytoplasm of the meniscal chondrocytes; 3) the expression levels of p38, NF-κB (p50/p65), and COX2 were significantly higher in

MD compared to non-MD (p<0.005 for all) and were positively correlated to each other and to the symptoms' duration (p<0.001 for all); 4) MD was significantly associated with knee OA (p=0.003).
Conclusions: 1) Constitutive expression of p38–NF-κB signalling cascade and COX2 may be implicated in MD pathobiology in a coordinated fashion; 2) Knee OA is related to the development of MD and teared meniscus; 3) Targeted disruption of the p38–NF-κB–COX-2 axis may constitute a novel approach to inhibiting OA progression, preventing meniscal degeneration and rapture.

63 Smooth Muscle Tumors of the Inguinal Canal in Women Are Separable into Two Clinicopathologically Distinct Groups

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Background: Assessment of the biologic potential of smooth muscle tumors (SMTs) relies on evaluation of multiple parameters. In this study, we examined SMTs from the inguinal region of women to define their clinicopathologic features.
Design: Fifty-five archived examples of SMTs from the inguinal region of women were evaluated for tumor size, cell-type, cytological atypia, mitotic activity (mitotic figures (MF)/10 HPFs), and presence of coagulation necrosis. Follow-up status was also recorded. Immunohistochemical (IHC) evaluation was performed on select cases with available material.
Results: Group (Grp) A (n=23, mean age, 49 yrs) tumors arose mostly in the round ligament (7/12 with known location) and had a mean size of 7.8 cm. Histologically, the SMTs resembled conventional uterine leiomyomas with well-circumscribed borders (14/22); minimal cytological atypia; low mitotic activity (mean-2.2 MF/10HPF); and no atypical mitosis or coagulation necrosis. Grp B (n=32, mean age, 61 yrs) tumors were located mostly in subcutaneous tissues (13/18 with known location) and had a mean size of 5.4 cm. These tumors exhibited cytological atypia; high mitotic activity (mean-40 MF/10HPF), atypical MF and necrosis. IHC profile for Grp A/B included expression of SMA (90%/100%); desmin (73%/60%); h-caldesmon (55%/57%); keratin (8%/0%); EMA (15%/33%); ER (83%/20%); PR (83%/25%); and WT-1 (50%/12%). Grp B ER/PR positivity was found in 5%-10% of cells; strong expression of both in 2 cases. Seven Grp A patients with follow-up (range, 2-29 yrs; median, 12 yrs) were alive and four died (unknown/unrelated causes). Grp B patient follow-up (range, 2-238 months; median, 48 months) included 17 deaths (1 of tumor; 16 of unknown/unrelated causes) and 3 living patients (disease status unknown).
Conclusions: Inguinal SMTs in women are a dichotomous group. Grp A tumors consist of well-differentiated, hormonally sensitive SMTs that commonly express WT-1, have a good prognosis, and resemble uterine leiomyomas. Grp B tumors are leiomyosarcomas that are usually WT-1 and ER/PR negative. All Grp A SMTs, and those Grp B SMTs that strongly express ER/PR (or WT-1) (n=2), are probably Mullerian-derived and should be classified according to established histologic criteria for subtyping uterine SMTs. In addition to hormone receptors, WT-1 serves as an additional marker of Mullerian derivation for inguinal SMTs.

64 Alveolar Rhabdomyosarcoma of the Head and Neck Region in Older Adults

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Background: Alveolar rhabdomyosarcoma (ARMS) is remarkably rare in adults over the age of 60. Initial immunoprofiling of a neoplasm with small cell morphology of the head and neck region in an older adult may not include muscle markers. A valuable diagnostic aid and important prognostic parameter in ARMS is the identification of translocations t(2;13)(q35;q14) and t(1;13)(p36;q14), and the associated *PAX3/FKHR* and *PAX7/FKHR* fusion transcripts respectively. The purpose of this study was to describe the clinicopathologic and genetic features of head and neck ARMS in older adults.
Design: The clinicopathologic features of 4 adult head and neck ARMSs referred for genetic analysis were reviewed. RT-PCR analysis for the identification of *PAX3/* or *PAX7/FKHR* fusion transcripts was performed on each case. Conventional cytogenetic and *FKHR* FISH analysis was also performed on one case.
Results: Patients included 3 females and 1 male, 61 to 76. Sites of involvement included: nasopharynx, hard palate, ethmoid and maxillary sinuses. Each neoplasm was composed of small round cells in a predominantly solid pattern. One case showed extensive crush artifact. Initially ordered immunostains corresponded with early diagnostic impressions of lymphoma or neuroendocrine carcinoma. Notably, tumor cells of all cases were CD56+ and 2 cases were also synaptophysin+. Due to virtual absence of other lymphoid or epithelial markers however, muscle markers were subsequently pursued and were uniformly positive. Molecularly, 3 cases demonstrated a *PAX3/FKHR* fusion transcript (confirmed by karyotypic and FISH studies in 1 case) and the remaining case a *PAX7/FKHR*.
Conclusions: These studies suggest establishing a diagnosis of adult head and neck ARMS is complicated by its exceptional rarity in this age group (>60 years old), lack of alveolar pattern in most cases, potentially misleading immunoprofile (CD56 immunoreactivity ± synaptophysin) and crush artifact in a subset of tumors. Notably, both *PAX3/* and *PAX7/FKHR* positive ARMSs were identified in this study. In the pediatric population, *PAX7/FKHR* positive ARMSs are associated with a significantly longer event free survival. In contrast, adult ARMS tends to behave more aggressively with a worse overall survival than pediatric ARMS. Further followup and additional cases will be required to assess the prognostic relevance of these fusion transcripts in the context of advanced age.

65 Expression of Insulin-Like Growth Factor II (IGF2) in Mesenchymal Tumors: An Immunohistochemical Study in 1100 Tumors

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Background: The insulin-like growth (IGF) factor system plays an important role in growth and development of cells and has recently been implicated in tumor development and progression. Gene expression profiling studies on limited numbers of specimens have demonstrated consistently high expression of *IGF2*, encoding the activating ligand for this system, in gastrointestinal stromal tumors (GIST) and synovial sarcomas. This data has may have concrete clinical implications, as several reports exist of GIST patients suffering from severe hypoglycemia, a predicted effect of IGF2. Furthermore, new drugs targeting IGF signaling are entering clinical trials. The purpose of this study is to survey IGF2 expression at the protein level on a broad number of mesenchymal tumors representing all major diagnostic classes, and correlate to outcome in GIST.
Design: Seven different tissue microarrays representing 1100 mesenchymal tumor cases were scored for IGF2 immunostaining. Immunoreactivity was considered positive in distinct membranous and/or cytoplasmic staining according to published criteria, scored as negative, focal (<10% of tumor cells positive), moderate (10-50%) or high (>50%). For statistical analysis the 'positivity cut-off' included only moderate and high positivity cases.
Results: Results were obtained for 54 diagnostic classes of bone and soft tissue tumors representing 1100 cases. Among those with at least 10 cases, only GIST, synovial sarcoma and dermatofibrosarcoma protuberans demonstrated high level expression in the majority of samples. Moderate expression was present in a minority of myxoid liposarcomas, leiomyosarcomas, rhabdomyosarcomas, angiosarcomas, nerve sheath tumors and undifferentiated sarcomas. IGF2 expression was rare or absent in lipoma, well-differentiated liposarcoma, fasciitis, fibromas, fibromatoses, giant cell tumors, Ewing sarcoma and chondrosarcomas. In GIST, the 278 cases with high expression of IGF2 had significantly worse outcome than the other 164 cases examined.
Conclusions: Among mesenchymal tumors, GIST, synovial sarcoma and DFSP are the best candidates for anti-IGF2 therapies.

66 Assessment of Muscarinic and Nicotinic Acetylcholine Receptor (AchR) Expression in Primitive Neuroectodermal Tumor (PNET)/Ewing's Family of Tumor (EFT) and Desmoplastic Small Round Cell Tumor (DSRCT): An Immunohistochemical Study of Tissue Microarray and Western Blot Study of Tumor Cell Lines

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Background: Neurocrest-derived tissues express muscarinic and nicotinic acetylcholine receptors (mAChR and nAChR respectfully). There is data demonstrating involvement of muscarinic and nicotinic acetylcholine system in neurocrest-derived malignancy as well as non-neurocrest-derived benign tissue and malignant tumors. However, little is known about the role of this pathway in sarcomas. In this study we evaluate primitive neuroectodermal tumors (PNET)/Ewing's Family of Tumor (EFT) and desmoplastic small round cell tumors (DSRCT) for the presence of mACh and nACh receptors.
Design: Forty-seven cases of PNET/EFT and two DSRCT archived at H, Lee Moffitt Cancer and Research Institute was retrieved by computer-assisted query. Tissue microarray was constructed consisting of 34 cases of PNET/EFT. We evaluated the presence of M3 and M5 mAChR as well as beta-2 and alpha-7 nAChR expression on TMA section of PNET/EFT and histologic section of DSRCT. The immunostains were analyzed by both computerized imaging analysis and manual examination. We also conducted western blot analysis on 3 Ewing sarcoma cell lines with M3, M5 and alpha 7 antibodies.
Results: M3 was immunoreactive in 31 of 34 cases (91%) and M5 was positive in 26 of 34 cases (77%) of PNET/EFT. Both of the muscarinic receptors were positive in 2 of 2 cases (100%) of DSRCT. Furthermore, beta-2 was not significantly immunoreactive in either PNET/ETF or DSRCT where as alpha-7 nAChR demonstrated positivity in 25% of the PNET/ETF. Western blot analysis of human PNET/EFT cell lines revealed distinct banding at the appropriate size for each receptor, confirming the immunohistochemical results.
Conclusions: M3 and M5 mAChRs as well as alpha-7 nAChR are expressed in a significant number of PNET/EFT and DSRCT. There are multiple new pharmaceuticals that specifically target these receptors and the signal transduction cascade associated with ligand binding. Future studies are planned to investigate the mechanism and potential therapeutic implications of these receptors.

67 Analysis of CHOP Rearrangement in Pleomorphic Liposarcoma Using Fluorescence In Situ Hybridization

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Background: Pleomorphic liposarcoma (PLS) is a rare aggressive subtype of liposarcoma composed of high-grade sarcoma with varying number of pleomorphic lipoblasts. PLS usually consists of heterogenous histological components and sometimes has myxoid or small round cell areas similar to myxoid/round cell liposarcoma (MLS/RC). Cytogenetically, PLS often possesses complicated karyotypic abnormality. Recent study have revealed FUS-CHOP fusion transcripts specific for MLS/RC in some cases of PLS by reverse transcription-polymerase chain reaction (RT-PCR) method.
Design: To investigate the existence of CHOP split signals in various histological areas including MLS/RC-like feature and also estimate the distribution of normal and some types of abnormal signals in PLS using FISH analysis. Seven PLS and three MLS/RC were selected for FISH analysis using LSI CHOP (12q13) dual color, break apart probe (Vysis, USA). FISH analysis was applied to formalin fixed, paraffin embedded

tissue sections of representative areas in all cases. We counted two hundred nuclei and estimated the percentage of nuclei with split signal, normal signal, and the other various types of abnormal signals.
Results: Six cases of seven PLS showed CHOP split signal ranged 0.5 to 3% of counted nuclei, while all cases of MLS/RC exhibited CHOP rearrangement over than 10% of counted nuclei. All cases of PLS showed various distribution of normal and abnormal signals in each histological areas.
Conclusions: A CHOP rearrangement in PLS should be recognized as only a representative part of complex karyotypic feature, because the number of cells with split signals was surely minute compared with that of MLS/RC, and the signals were found in any areas despite of its histological difference. Therefore, we have to carefully estimate the association between the results of FISH analysis and histological subtypes with a characteristic rearrangement.

68 The Metastatic Dichotomy between Sarcomas and Carcinomas Is Partially Explained by Their Relative Levels of Vasculogenesis and Lymphangiogenesis but Not by Their VEGF Expression Profile

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Background: Human carcinomas are known to metastasize primarily through lymphatics to lymph nodes whereas sarcomas with few exceptions spare the lymphatics and metastasize hematogenously to visceral organs. However the reason for this metastatic dichotomy is not understood. With the newly emerging experimental evidence that metastasis may be regulated by local vasculogenesis *v* lymphangiogenesis, we wondered whether these might differ in sarcomas *v* carcinomas and account for their metastatic dichotomy.
Design: We decided to examine 20 sarcomas and 20 carcinomas to assess their comparative degrees of vasculogenesis *v* lymphangiogenesis. We conducted IHC studies with lymphatic (D2-40), vascular (CD31) and proliferation (Ki-67) markers singly and in combination, the latter employing a dual chromogen technique designed to determine the relative levels of vasculogenesis *v* lymphangiogenesis and vascular *v* lymphatic invasion. We also studied the expression profile of the vascular endothelial growth factor (VEGF) family members: VEGF-A, -B, -C, -D in these tumors by RT-PCR and real time PCR.
Results: In all the sarcomas and carcinomas studied, four vascular populations were in evidence: D2-40 lymphatics with and without tumor emboli; CD31 blood vessel capillaries with and without tumor emboli. The D2-40 lymphatics were more numerous in the carcinomas ($p<.001$) and the CD31 blood vessels were more numerous in the sarcomas ($p<.01$). The sarcomas showed more vascular invasion ($p<.01$) than the carcinomas which exhibited greater lymphatic invasion ($p<.01$). While a greater percentage of the carcinoma D2-40 lymphatics showed proliferation ($p<.01$), a greater percentage of the sarcoma CD31 vessels showed proliferation ($p<.05$).VEGF-A, -B, -C and -D transcripts were detected by RT-PCR in all of the sarcomas and carcinomas but by real time PCR the sarcomas surprisingly had 25-50 fold greater VEGF-C whereas the carcinomas had 3 fold greater VEGF-A. VEGF-B, -D were low in both groups.
Conclusions: These studies suggest that sarcomas, in contrast to carcinomas, stimulate minimal lymphangiogenesis but maximal vasculogenesis. But this effect is not mediated by the relative levels of their VEGF family members because VEGF-C which is thought primarily to stimulate lymphangiogenesis is paradoxically high in sarcomas This suggests that the mechanism behind the differential vasculogenesis / lymphangiogenesis in sarcomas needs to be elucidated.

69 Differential Expression of the Oncoprotein c-Jun in Liposarcomas Highlights Tumors of Dedifferentiated Type

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Background: A recent report demonstrated that c-Jun amplification and overexpression is a distinguishing characteristic of a subset of aggressive sarcomas, which appear to represent dedifferentiated liposarcomas (DDLPS). Overexpression of c-Jun was also shown to block adipocytic differentiation in a model system (Mariani et al., Cancer Cell 2007). However, only limited data are available on the relative levels of c-Jun protein in liposarcomas with both well-differentiated (WD) and dedifferentiated (DD) components.
Design: We performed immunohistochemistry for c-Jun in a series of liposarcomas (DDLPS, well differentiated (WDLPS), myxoid and pleomorphic) derived from central, peripheral and metastatic sites. The intensity of c-Jun staining (from 0 (no staining) to 3+ (strong staining)) was scored independently by 2 pathologists.
Results: The majority of DDLPS exhibited moderate to strong c-Jun staining (average intensity = 2+; n=23). In contrast, c-Jun levels in pure WDLPS were low (average intensity = 0.6+; n=5). In those cases of DDLPS with a WD component, average c-Jun levels were lower in the WD component than the DD component (average intensities = 1.8+ for DD and 1.3+ for WD components; n=18; $p<0.03$), but higher than those seen in pure WDLPS ($p<0.03$). We also found that c-Jun levels were low in all cases of cytogenetically confirmed myxoid (average intensity= 0.4+; n=5) and pleomorphic (average intensity= 1+; n=2) liposarcomas.
Conclusions: We find that c-Jun, an inhibitor of adipocytic differentiation, is generally expressed at higher levels in DDLPS than WDLPS. However, the WD components of DDLPS often express higher c-Jun levels than pure WDLPS. We hypothesize that WD components with moderate c-Jun protein expression may represent a biologically intermediate state between DDLPS and pure WDLPS and that c-Jun immunopositivity in WDLPS may potentially be a harbinger of concurrent or incipient dedifferentiation. Additional studies are underway to determine the genetic basis of differential c-Jun expression in these tumors, as well as its expression in other sarcoma types.

70 Fluorescence In-Situ Hybridization (FISH) Is a Useful Ancillary Diagnostic Tool for Extraskeletal Myxoid Chondrosarcoma

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Background: Extraskeletal myxoid chondrosarcoma (EMC) is a rare soft tissue sarcoma of uncertain differentiation. EMCs are typically characterized by a nodular growth pattern with reticular strands of eosinophilic cells with abundant myxoid stroma and can cause confusion with other myxoid sarcomas. Immunohistochemistry is usually non-specific. The majority of EMCs harbor a balanced t(9;22)(q22;q12) that fuses *EWSR1*with *NR4A3* (aka *CHN*). Other less common variant translocations involving *NR4A3* have also been described. We examined the diagnostic utility of FISH using an *EWSR1* break-apart DNA probe on formalin-fixed paraffin-embedded (FFPE) tissue for EMC.
Design: Eleven cases of EMC with FFPE tissue available were retrieved from the pathology files of our institution from 1990-2007 and clinical information obtained with prior IRB approval. Unstained coated slides were prepared and FISH was performed using the LSI *EWSR1* break-apart probe set (Vysis, Downers Grove, IL).
Results: The median age at presentation was 54 (30-73) years. There were 9 males and 2 females. All 11 cases were either consistent with or highly suggestive of the diagnosis though one case exhibited higher grade features. All eleven tumors occurred in the thigh, inguinal or gluteal region. Ten cases were analyzable by FISH of which nine (including the higher grade case) were positive for rearrangement of the *EWSR1* locus.
Conclusions: Nine of the ten analyzable EMCs contained a rearrangement at the *EWSR1* locus (22q12) detected by break-apart FISH probes. This underscores the prevalence of *EWSR1* rearrangements over the other described alternative translocations. FISH is effective in the diagnosis of EMC in most cases, and can differentiate it from other myxoid sarcomas lacking this rearrangement.

71 Identification of the ASPL/TFE3 Fusion Transcript and Immunohistochemical Detection of TFE3 in Formalin-Fixed Paraffin-Embedded Tissue: Their Role in the Diagnosis of Alveolar Soft Part Sarcoma (ASPS)

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Background: ASPS is a rare mesenchymal malignancy. Its diagnosis can be problematic due to histological overlap with other tumours as well as a lack of specific diagnostic markers. ASPS has a recently described unbalanced translocation, der(17)t(X;17)(p11;q25), with type 1 and 2 variants involving fusion of the first seven exons of ASPL to exon 6 (type 1) or 5 (type 2) of TFE3 (GenBank NM_006521). Anti-TFE3, a commercial antibody, recognizes the carboxy terminal portion of TFE3, resulting in strong nuclear staining.
Design: RNA was extracted from 13 formalin-fixed, paraffin-embedded cases of typical ASPS. Novel primers were designed to detect type 1 and type 2 fusions to produce PCR products of 120bp and 130bp, respectively. RT-PCR for both fusion transcripts was performed on 13 ASPS as well as 20 negative controls, including other sarcomas, metastatic carcinoma, melanoma and granular cell tumour. All PCR products were confirmed by DNA sequencing. Immunohistochemistry was carried out on 4μm paraffin sections of all samples using an anti-TFE3 goat polyclonal antibody (Santa Cruz Biotechnology) with the avidin-biotin peroxidase technique.
Results: RNA was successfully extracted from all 13 ASPS and 20 controls. All 13 ASPS contained a fusion transcript; 8 were Type 1 and 5 were Type 2. All 13 ASPS had strong nuclear immunostaining in at least 50% of the tumour cells. Four granular cell tumours, including a malignant one, showed variable degrees of nuclear staining; all of these were negative for the fusion transcript. All other tumours tested were negative with anti-TFE3.
Conclusions: 1) Anti-TFE3 is a sensitive marker, but not entirely specific for some of the simulators of ASPS. 2) RT-PCR techniques, designed for application on paraffin-embedded material, are highly sensitive and specific in detecting both ASPL/TFE3 fusion transcripts. 3) RT-PCR verification of immunopositive cases that are not entirely characteristic of ASPS histologically is recommended.

Breast

72 D2-40: An Additional Marker for Myoepithelial Cells of Breast

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Background: D2-40 is a recently available mouse monoclonal antibody specifically recognized human podoplanin and has been used in identifying lymphovascular invasion of tumors. Although its expression has been evaluated in other tissues, its use as a marker of the myoepithelial cells of breast has not been reported. We have found that it serendipitously stains the myoepithelial cells of the terminal duct lobular units, and its utility in breast pathology is therefore further explored.
Design: Histopathological materials of 48 patients with a variety of breast diseases were reviewed and paraffin embedded tissue blocks were chosen to include usual ductal hyperplasia (41 cases), atypical ductal hyperplasia (5 cases) and ductal carcinoma in situ (DCIS, 17 cases) for this study. Normal breast parenchyma and invasive carcinoma were also noted in some of the tissue sections. Immunohistochemistry for D2-40, p63 and Calponin was performed and the results were compared.
Results: D2-40 immunohistochemistry stains the cytoplasm of the myoepithelial cells along the periphery of benign proliferative lesions, atypical ductal hyperplasia and majority of DCIS, and in the luminal lesions of usual ductal hyperplasia. The staining pattern is identical to that of Calponin with less intensity. In addition the staining of