Conclusions: Our findings provide compelling morphologic evidences that (a) the kidney is the primary target in BMT-associated TMA and (b) morphologic findings strongly correlate with the clinical diagnosis of TMA. Our study did not identify a significant etiologic relationship between BMT-TMA and AFI or other clinical variables. The findings are suggestive of multifactorial etiology and complex pathogenesis in BMT-associated TMA.

28 Myocarditis in the Autopsy Heart: Retrospective Analysis of a Major Urban Medical Examiner Case Population

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Background: The histologic pattern of myocarditis in cases of sudden death may be different from the histologic criteria used to diagnose myocarditis by endomyocardial biopsy in clinical practice. Much of the literature regarding the histologic diagnosis of myocarditis refers to findings on endomyocardial biopsy of symptomatic patients. The significance of focal and diffuse myocarditis discovered during the autopsy of individuals who die suddenly and unexpectedly has not been well defined. This study is a retrospective analysis of myocarditis identified at autopsy in an urban (Chicago) medical examiner's office.

Design: All cases with the autopsy diagnosis of myocarditis in the records of the Office of the Medical Examiner, Cook County, IL from 1998-2003 were identified. The medical examiners investigative report, autopsy protocol, toxicology and laboratory results and available medical records were reviewed. Cases were analyzed for the presence of co-morbidities such as significant disease, injury or drug intoxication and for a diagnosis of diffuse or focal myocarditis .

Results: Of 27,000 autopsies performed by the Cook County Medical Examiners Office in that period, there were 84 cases diagnosed with myocarditis. There were 52 adults (>18 years) with males equal to females and 32 were children with 17 males and 15 females. Eleven (11) of the children were under one year of age and 8 of these were male (p=0.001). Seventeen (17) of the 23 children with diffuse myocarditis had no co-morbidity while 2 of 9 with focal myocarditis had no co-morbidity (p=0.0147, Fishers exact test). Eleven (11) of 17 adults with diffuse myocarditis had no co-morbidity whereas only 1 of 34 with focal myocarditis was without a significant co-morbidity (p=0.0001 Fishers exact test). There was no statistical difference in co morbidity for age (children vs. adults) for diffuse (p=0.73) or focal (p=0.10) myocarditis.

Conclusions: Children dying with diffuse myocarditis were more likely to have no co-morbidities related to their deaths and those with focal myocarditis were more likely to have significant co-morbidities. In adults diffuse disease was associated more often with no co-morbidities but focal disease was almost exclusively associated with co-morbidities. These results question the significance of focal myocarditis in this autopsy population.

29 Differential Expression of INOS, VEGF and COX-2 in the Lungs Is Related to Pulmonary Hypertension in Sickle Cell Disease: An Autopsy Study of 23 Cases

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Background: Approximately 40% of patients with sickle cell disease (SCD) develop pulmonary hypertension (PH). The pathogenesis of SCD-associated pulmonary hypertension is unknown; hemolysis, chronic anemia, and frequent transfusions are significant predictors of PH. Reduction in NOS and increased expression of VEGF is reported in PH and in plexiform lesions. COX-2 is a mediator of angiogenesis. We investigated the expression of INOS, VEGF and COX-2 in the pulmonary arteries of autopsied patients with SCD to explore the pathogenesis of PH.

Design: Paraffin-embedded lungs from 23 autopsied adults (14 males and 9 females) were immunostained using enhanced sensitivity avidin-biotin peroxide method, and antibodies against nitric oxide synthase (INOS), vascular endothelial growth factor (VEGF), and cyclo-oxygenase -2 (COX-2). Clinical data were collected for correlation with pathologic data. Immunostained slides were graded: 0= 0, 1=weak, and 2= strong. Pulmonary vasculature endothelium and smooth muscle, and airway epithelium and smooth muscle were graded. Statistical analysis of the data was performed using Fisher's Exact test and Chi-Square.

Results: Mean age was 41 years (14 M=35, 9 F= 51). Hemoglobin S fraction percentages ranged from 23% to 98%; 9 patients had sickle cell trait (SCT) with HgbS fraction <40%;14 had SCD (HgbS >40%). All 23 cases showed PH grade I to grade IV (plexiform lesions in 57%). INOS expression was weak in the vascular endothelium (21%) but strong in smooth muscle (57%), and weak to moderate in the airways. VEGF expression was strong (85-100%) in vascular endothelium and smooth muscle (70-100%), but weak to moderate in the airways. COX-2 expression was absent to weak in both vessels and airways. In addition, more males had severe SCD compared to females; and more males than females had the diagnosis of sudden death (43% vs 11%). Cardiomegaly was present in 22 / 23 patients (mean weight=498 gms); mean weight of those with SCT (474 gms) was lower compared to SCD (514 gms).

Conclusions: Histologic changes of PH are present in all patients with SCH at autopsy. The weak expression of INOS in the endothelium and strong expression in the muscle suggests possible etiologic role of INOS in SCD-associated PH. The strong expression of VEGF in the vessels may be the cause of the muscular and endothelial hyperplasia and PH. Lack of COX-2 expression indicates that cytokines are not involved in the pathogenesis of PH in SCD.

Bone & Soft Tissue

30 The Prognostic Significance of Fibrosarcoma Arising in Dermatofibrosarcoma Protuberans

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Background: Dermatofibrosarcoma protuberans (DFSP) is a superficial tumor characterized by high rates of local recurrence and a low risk of metastases. Fibrosarcomatous (FS) areas rarely arise in DFSP and considerable controversy exists as to whether these tumors have a higher risk for metastases than typical DFSP. The aim of this study was to reappraise the prognostic significance of FS change in DFSP.

Design: The consultation files of our institution revealed 41 patients with fibrosarcoma arising in DFSP and the clinicopathologic features of each case were analyzed retrospectively. The tumors were examined for the proportion of FS areas, mitotic count, CD34 expression (0 to 3+), MIB-1 labeling index (LI), and p53 expression. Follow-up was obtained in 38 cases (median: 48 mo, range: 1-300 mo). Prognostic variables for predicting local recurrence were evaluated using a univariate Cox model.

Results: The study included 23 females and 18 males with a median age of 48 yrs (range: 16-100). Eighteen lesions were seen on the trunk, 16 on the extremities, and 7 on the head/neck. All tumors were treated with wide local excision and the surgical margins were considered positive in 22 of 39 cases (56%). Fibrosarcoma arose de novo in 38 cases and as a recurrence in 3 cases. All tumors involved the dermis and subcutis and the FS component comprised 5-95% of tumor area (median: 60%). Mitotic rates of the FS component (20/10hpf, range: 5-48) was considerably higher than neighboring DFSP (0-3/10hpf). CD34 expression was stronger and more extensive in the DFSP component (97%, median intensity 3+) than in the FS component (81%, median intensity 2+). Diminishment of CD34 staining was seen in the FS areas (median intensity 0/1+) in 15 cases. The MIB-1 LI of the FS regions was higher (median, 20%; range: 5-45%) than the DFSP areas (<3%). p53 expression was gained in the FS areas (92% positive) versus the adjacent DFSP (3% positive). Follow-up data revealed local recurrences in 8 patients (5-year local recurrence free survival, 68%), metastases in 4 patients (10%), and 3 patients died of disease. None of the variables evaluated (including margin status, FS area, or mitotic rate) correlated with disease progression.

Conclusions: Fibrosarcoma arising in DFSP is a form of tumor progression that carries a small, but definite risk for metastases. The pathogenesis of this progression can be partially explained by gains of *p53* mutations. In patients with FS transformation of DFSP, neither the extent of the FS area, mitotic rate, nor the margin status correlated with disease progression.

31 Deep-Seated Plexiform Schwannomas (PS). A Pathologic Study of 16 Cases and Comparative Analysis with the Superficial Variety

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Background: PS is the least common variant of schwannoma and typically occurs in the dermis and subcutaneus tissue. Morphologically, PS can display a conventional, cellular or mixed appearance. However, the frequent cellular morphology associated with hyperchromatic nuclei, increased mitoses, and plexiform growth can suggest a malignant process, mainly a high-grade malignant peripheral nerve sheath tumor (MPNST). Our objective was to study the clinicopathologic features of deep-seated PS, and compare them with the more common superficial variety.

Design: Clinicopathologic, immunohistochemical (IHC) and ultrastructural features from 16 deep PS were analyzed and compared with 8 superficial (5 dermal and 3 subcutaneous) PS. None had stigmata of neurofibromatosis.

Results: The deep PS occured in 12 females and 4 males. 15 PS were located in the deep somatic soft tissue (pelvis/retroperitoneum, 4; extremity, 8; trunk, 2; parotid, 1) and 1 in the viscera (thoracic esophagus). The largest tumor was 15 cm in size. The tumors frequently showed increased cellularity (68%), mild-moderate nuclear pleomorphism (50%) and mitotic activity (93%). Focal necrosis was seen in 12% and myxoid changes in 18% of cases. Verocay bodies were identified in 62% of cases. IHC (S100 protein, Laminin) and ultrastructural analysis were consistent with a well-differentiated schwannian proliferation in all cases analyzed. The 8 superficial PS occured in 5 males and 3 females and were located in the dermis and subcutis (lower extremity, 5; shoulder,1; perianal region,1). The tumors showed increased cellularity (62%), mild to moderate pleomorphism and mitotic activity (62%). No necrosis was identified. Verocay bodies were identified in 62% of cases.

Conclusions: Deep-seated PS is a rare, under-recognized PNST, typically not associated with neurofibromatosis. Although frequently occurring in the extremities, they can be seen in other locations such as viscera and can grow up to 15 cm in size. In contrast with the more common superficial tumors, deep PS have predilection for females, can occur in congenital settings, and can show necrosis and myxoid change. However worrisome histologic features were seen in both groups, including increased cellularity, mild to moderate pleomorphism and mitoses. It is important to differentiate these tumors from plexiform neurofibromas and MPNST, as they follow a benign clinical course, with complete surgical excision being curative.

32 A Simple and Reliable Method for the Molecular Diagnosis of Fibrous Dysplasia

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Background: Fibrous dysplasia (FD) is a benign intra-medullary fibro-osseous lesion, resulting from missense mutations in the GNAS1 gene. Distinguishing between FD and other pathological processes is not always easy and a robust molecular diagnosis would help in difficult cases. Known GNAS1 mutations occur in codons 201(C→T

or G→A) and 227(A→T). We have compared the number of mutations detectable using a site-directed mutagenesis approach and direct DNA sequencing in extra-gnathic sites. We have also assessed the number of GNAS1 mutations in gnathic FD and non-FD fibro-osseous lesions (cemento-ossifying fibroma=COF and florid cemento-osseous dysplasia=FCOD).

Design: 46 extra-gnathic (mono and polystotic) and 16 gnathic FD, 24 FCOD and 29 COF cases were studied. DNA was extracted from micro-dissected paraffin-embedded sections from decalcified specimens. Using a site-directed mutagenesis approach, 3 PCR reactions for each case were performed to detect the 3 known mutations. The use of mismatch primers followed by digestion with the appropriate enzyme (Nco1, Dde1 or Stu1) discriminates between the wild-type allele (undigested) and mutant allele (digested). DNA from the extra-gnathic FD cases was also analysed by direct sequencing.

Results: PCR products were generated from 46 extre-gnathic FD cases for the site-directed mutagenesis method and from 39 cases for sequencing, from 13 of 16 FD gnathic cases and from 22 and 20 of FCOD and COF cases, respectively. 31 mutations (67%), including 1 A→T mutation, were detected in extra-gnathic FD lesions, while 23 mutations (59%) were detected using direct sequencing. Sequencing failed to detect mutations in 6 cases. Therefore, the site-directed mutagenesis method showed a 15% improvement in mutation detection. The mutations detected by both methods were always identical. Mutation detection did not reflect the cellularity of an FD lesion, but was increased by analysing more than 1 tissue block. Mutations were detected in 5 (38.5%) gnathic FD cases, including 1 A→T mutation, 2 FCOD and 1 COF cases.

Conclusions: In the largest study of its kind, we present a simple and reliable site-directed mutagenesis method for the molecular diagnosis of FD that shows a 15% improvement in the rate of mutation detection compared with direct sequencing. We report the first two codon 227 mutations detected in fibrous dysplasia. Our results provide evidence that the pathogenesis of FCOD and COF is different to FD.

33 Acquired Resistance to Imatinib in Gastrointestinal Stromal Tumor (GIST) Occurs through Secondary Gene Mutation

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Background: In up to 90% of cases, GISTs have activating mutations in either *KIT* or *PDGFRA*, both of which encode class III receptor tyrosine kinases. Imatinib mesylate specifically inhibits KIT, PDGFRA, BCR-ABL, and ABL. Initially applied in the treatment of CML, imatinib was then tested in metastatic/unresectable GIST and found to achieve a partial response or stable disease in over 80% of patients. More recently, the first signs of imatinib–resistance in GIST are being seen. Our objective was to identify mechanisms of acquired resistance to imatinib in GIST in order to set out strategies to prevent or delay its development.

Design: 31 patients with the diagnosis of GIST who were treated with imatinib and underwent surgical resection of their tumor were included in the study. Based on the responsiveness of their disease to imatinib at the time of surgery, we categorized patients as "non-resistant" (n=14), "primarily resistant" (n=3), or "secondarily resistant" (n=15). From these 31 patients, 63 tumor nodules were available for assessing histologic response, *KIT/PDGFRA* genotyping, and KIT phosphorylation (P-KIT) by western blotting.

Results: In the non-resistant group, 6/14 had a histologic response of >90%. There were no identifiable second mutations in this group. 4 cases showed a moderate or strong P-KIT expression. All but 2 patients with acquired resistance had at least 1 nodule lacking histologic response. Secondary mutations were seen in 11 samples from 7 (46%) patients with acquired resistance. The most common site was *KIT* exon 17 (n=6), and included N822K (n= 3), D820Y (n=2), and Y823D (n=1). In 2 patients, the 2nd mutation was present on one but not all nodules. Although P-KIT was detected in 12/13 resistant patients tested, only 4 (30%) showed a strong activation. The 3 primary resistant patients lacked secondary mutations or histologic response.

Conclusions: Resistant GIST show common secondary mutations in the KIT kinase domain, which are single amino-acid substitutions and are present on the same allele with the primary mutation, typically located in the *KIT* exon 11. Secondary mutations were not seen in any of the pre-imatinib, non-resistant or primary resistant samples tested. Significant heterogeneity was identified at the KIT protein activation and histologic response among different resistance groups.

34 Expression Profiling of Tyrosine Kinase Genes in Synovial Sarcoma Reveals a Highly Distinct Kinase Repertoire and Prominent Overexpression of PDGFRA

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Background: Protein kinases have a central role in cellular signaling and, as a group, are particularly rich in drug targets. The main mechanisms of pathological kinase activation are ligand-dependent overexpression of unmutated receptors and ligand-independent constitutive activation resulting from gain-of-function mutation, translocation or gene amplification. The kinase repertoires of different sarcomas have not been systematically examined.

Design: We used Affymetrix U133A GeneChip microarray data from 48 samples of synovial sarcomas (SS) (46 tumors and 2 cell lines) and 106 samples of 4 other types of translocation sarcomas (ES, ARMS, DSRCT, ASPS). We identified 739 probe sets on U133A chip corresponding to 432 kinase genes, representing 83% of the 518 known protein kinase genes identified in the human genome. We then performed unsupervised and supervised bioinformatic analyses limited to this subset of genes. Selected kinase genes were validated by IHC and were examined for somatic mutations by PCR and direct sequencing.

Results: Unsupervised hierarchical clustering based only on these 739 probe sets recapitulated almost perfectly the separation of SSs from the 4 other sarcoma types obtained using all probe sets on the U133A chip. The hierarchical clustering dendrogram showed no orphan SS samples, indicating that the repertoire of kinase genes expressed in this sarcoma is fundamentally distinct from that of ES, ARMS, DSRCT, and ASPS. PDGFRA was the kinase gene most specifically and highly overexpressed in SS. EGFR, previously reported as overexpressed in SS, was only weakly differentially expressed in SS in this dataset. Immunoreactivity for PDGFRA (1 to 3+) was observed in a subset of synovial sarcomas. Four of 7 cases with the highest PDGFRA gene expression showed moderate to strong (2 to 3+) membranous and cytoplasmic staining by IHC, more intense than a PDGFRA-mutated GIST control. Mutation screening of the kinase domains of PDGFRA (23 cases, 2 cell lines) and EGFR (5 cases) has so far been negative.

Conclusions: Our data show a highly tumor-specific pattern of protein kinase expression in sarcomas, especially in SS. PDGFRA is found to be strongly overexpressed in SS and a subset of cases show strong (2+ to 3+) immunoreactivity for PDGFRA. Further evaluation of the effects of PDGFRA targeting by Gleevec in certain SS may be warranted.

35 Epithelioid Hemangioendothelioma of Bone - The Rizzoli Experience

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Background: The new WHO classification of bone tumors recognized epithelioid hemangioendothelioma (EpHE) as a low-grade malignant vascular tumor with the following histologic features: predominantly occult vascular spaces with epithelioid endothelial cells frequently containing intracytoplasmic vacuoles arranged in a chondromyxoid matrix. The features are identical to the tumors of soft tissue, lung and liver. EpHE of bone is rare. There are only a few case reports and small series in the literature. We reviewed all cases of EpHE seen at the Rizzoli Institute in order to further evaluate the clinicopathologic and radiographic features of this uncommon tumor.

Design: In a review of 150 cases of malignant vascular tumors in the files of the Rizzoli Institute, we found 11 which fulfilled the histologic criteria for EpHE. Radiographs were available on 6 cases. Clinical information was obtained from clinical charts and correspondence with patients and their physicians.

Results: The 11 patients consist of 5 males and 6 females ranging in age from 17 to 79 years (mean 39 years; median 34 years). Pain and/or swelling were the main symptoms. The bones involved were: long bones (7), small bones of the foot (2) and vertebra and pubis (1 each). Three patients had radiographic evidence of multicentric disease. Of these 3 patients, one had involvement of the femur, tibia, calcaneus and soft tissue (thigh); one had lesions in the femur and tibia; and the other had lesions in the scaphoid and cuneiform. Radiographically, all of the tumors had an aggressive appearance. Information on treatment and follow-up was available for 7 patients. Five were treated with curettage and 3 of these had postoperative radiation. The remaining 2 developed local recurrence. One patient was treated with excision and developed recurrence managed with re-excision and radiation. One patient treated with resection was free of disease and local recurrence at 193 months. None of the patients were reported to have visceral involvement. Follow-up time ranged form 18 to 192 months (average 82 months). None of the patients developed metastases or died of disease.

Conclusions: EpHE is a distinct histologic variant of low-grade malignant vascular tumors that have a tendency for local recurrence. Irradiation may be helpful in controlling local disease. Surgical management should be conservative.

36 Hemangiomas of Bone Previously Misclassified as Hemangioendothelioma

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Background: Recent studies have suggested that at least some hemangiomas of bone are being over-diagnosed as hemangioendothelioma and therefore over-treated. In order to further assess this issue as it applies to the bone files at the Rizzoli Institute, we reviewed the histologic features of 155 cases previously coded as malignant vascular tumors of bone.

Design: Four pathologists studied 155 cases without knowledge of the clinical follow-up. A diagnosis of hemangioma was made if the tumor consisted of well-formed vascular spaces (without anastomosis) lined with a single layer of flattened endothelial cells. A diagnosis of epithelioid hemangioma (EH) was made when the tumor had a lobulated growth pattern and was composed of plump cells lining well-formed vascular spaces and occasionally forming solid sheets. The endothelial cells contained vesicular nuclei without atypia, abundant eosinophilic cytoplasm and rare mitotic figures. Radiographs were available for review on 12 cases.

Results: There were 12 EH and 7 hemangiomas. The 19 patients consisted of 14 males and 5 females, ranging in age from 16 years (yrs) to 74 yrs. (mean 42 yrs; median 40 yrs). Nine tumors involved the long bones, 8 involved the flat bones and 2 involved the small bones of the feet. Five tumors were multifocal and 4 of these 5 were EH. Three of the 4 multicentric EH were centered around the ankle and foot. The remaining multicentric case was centered around the knee. Radiographically, all of the tumors had an aggressive appearance. Information on treatment was available for all patients and follow-up information was available for all but 2 patients. Fifteen patients were treated by curettage and 8 of these also received radiation therapy. Four patients were treated with resection without radiation. The average follow-up time ranged from 17 to 202 months (average 88 months). None of the 17 patients developed recurrent disease or metastases.

Conclusions: Benign vascular lesions, especially EH, may be mistaken histologically and radiographically for low-grade hemangioendothelioma. Careful attention to histologic criteria will help avoid a mistaken diagnosis. Surgical management of these tumors should be conservative.

37 Nuclear ß-Catenin Expression Distinguishes Deep Fibromatosis from Other Benign and Malignant Fibroblastic and Myofibroblastic Lesions

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Background: Deep fibromatoses (desmoid tumors) are clonal myofibroblastic proliferations that are prone to aggressive local recurrences but that do not metastasize. They must be distinguished from a host of fibroblastic and myofibroblastic lesions as well as from smooth muscle neoplasms. Virtually all deep fibromatoses have somatic ß-*catenin* or *adenomatous polyposis coli (APC)* gene mutations leading to intranuclear accumulation of ß-catenin. Since low-grade sarcomas in general lack ß-catenin and since reactive proliferations would not be expected to have it, we predicted that nuclear ß-catenin expression would be detected in deep fibromatoses but absent in other entities in the differential diagnosis. We evaluated the role of ß-catenin to help differentiate distinguish deep fibromatoses from congeners.

Design: Formalin-fixed paraffin embedded sections 20 cases of deep fibromatoses were stained with monoclonal ß-catenin antibody (Transduction Laboratories) and compared to low grade fibromyxoid sarcoma (n = 12), leiomyosarcoma (n = 10), various other fibrosarcoma variants (n = 13, including 3 myofibrosarcomas, 3 sclerosing epithelioid fibrosarcomas, 5 low grade fibrosarcomas, one classic fibrosarcoma arising in dermatofibrosarcoma protuberans, one inflammatory myxohyaline tumor/ myxoinflammatory fibroblastic sarcoma), myofibroma/myofibromatosis (n = 12), nodular fasciitis (n = 12), and scars (n = 11). Nuclear and cytoplasmic staining was assessed.

Results: All 20 examples of deep fibromatosis displayed nuclear ß-catenin (focal nuclear staining in one case to 90% staining; 18/20 displayed readily identifiable diffusely distributed nuclear staining). All other lesions tested (n = 70) entirely lacked nuclear labeling for ß-catenin, showing only cytoplasmic staining.

Conclusions: ß-catenin immunohistochemistry separates deep fibromatoses from a wide range of entities in the differential diagnosis, a finding that can be exploited for diagnosis. Most fibromatoses have diffuse nuclear staining although occasional examples only focally label.

38 SYT-SSX Fusion Transcripts in Synivial Sarcoma (SS) of Unusual Sites

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Background: SS is a highly malignant soft tissue tumor and accounts for up to 10% of soft tissue sarcomas. Histologically, SS is monophasic with spindle cell component or biphasic with both epithelial and spindle cell ones in varying proportion. SS arises mostly in deep soft tissue of extremities over 80% with predilection for young adults. Even though SS is a morphologically distinct entity, diagnosing SS is a challenging task if it is from unusual sites. A specific chromosomal translocation t(X;18)(p11.2,q11.2) results in the fusion of the SYT gene on chromosome 18 and SSX gene on chromosome X and has been well documented in SS. The chimeric gene transcripts have been detected in more than 90% of SS and considered as a sensitive and specific molecular marker for SS. To assess the diagnostic utility of the SYT-SSX fusion transcripts in SSs of unusual sites, the expression of the transcripts was studied.

Design: We analyzed clinicopathologic features of 21 SS patients at AMC from Jan. 1991 to Aug. 2004. Reverse transcriptase-polymerase chain reaction for SYT-SSX fusion transcripts was performed in all cases using formalin-fixed paraffin-embedded tissues.

Results: Patients included 14 women and 7 men with the age range of 18 - 73 years (mean, 41 years). The SS occurred most frequently in the extremities in 12 cases (57%). The others were from unusual sites, one each from pericardium, lung, thyroid, neck, oropharynx, chest wall, back, pelvic cavity, and kidney. Histologically, 14 cases (67%) were monophasic and 7 cases (33%) were biphasic. Three cases of monophasic SS were poorly differentiated ones with areas of high cellularity, numerous mitoses and often necrosis. The SYT-SSX fusion transcripts were identified in 19 cases (90%), including 11 cases (85%) of monophasic SS and all biphasic SS. The two SYT-SSX-negative cases were one poorly differentiated monophasic SS from lower extremity of 19-year-old woman and another conventional one from back of 55-year-old woman. Except for the back SS, all SSs from unusual sites expressed the SYT-SSX fusion transcripts.

Conclusions: These results indicate that molecular studies for SYT-SSX fusion transcripts are feasible on formalin-fixed paraffin-embedded tissues and also demonstrate that the SYT-SSX fusion transcripts are expressed in both monophasic and biphasic SSs regardless of sites. Therefore, molecular studies for the SYT-SSX fusion transcripts could be useful diagnostic tools particularly for the diagnosis of SSs of unusual sites.

39 Estrogen Signaling Is Active in Chondrosarcoma; Implications for Anti-Estrogens as Treatment of Chondrosarcoma

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Background: Chondrosarcoma is a malignant chondroid matrix producing tumour which can be lethal in 10-50% of the patients, correlated with histological grade. Often mutilating surgery is the only effective treatment known. These tumours are notorious refractory to all types of chemo- or radiotherapy. Sex steroids, especially estrogen, are important in the regulation of longitudinal growth that results from chondrocyte proliferation and differentiation in the epiphyseal growth plate of long bones. Both the accrual of bone mass during pubertal growth spurt and the closure of the growth plate that finalizes longitudinal growth are regulated by estrogen. Estrogen is produced through the activity of the enzyme aromatase. The estrogen receptor transduces the estrogen mediated signal to nucleus. This study investigates whether in chondrosarcomas estrogen signaling is active. This is in analogy with breast cancer, where estrogen mediated processes are targets for therapy.

Design: With immunohistochemistry we have studied 21 chondrosarcomas for protein expression of the estrogen receptor and looked at the mRNA levels using quantitative PCR for the estrogen receptor and for aromatase, a crucial enzyme for estrogen synthesis and another potential therapeutic target. Furthermore the activity of aromatase was determined *in vitro* by the tritiated water release assay. Cell cultures were confirmed to be tumour derived, by RNA expression of cartilage specific genes, Col10A1, Aggrecan and SOX 9 and by an aberrant karyotype.

Results: All chondrosarcomas tested showed mRNA and nuclear protein expression of the estrogen receptor indicating that the receptor is activated. Also aromatase mRNA was detected. Aromatase activity showed that 5 out of 6 primary chondrosarcoma cultures and 1 out of 2 established chondrosarcoma cell lines have a functional aromatase enzyme.

Conclusions: These results indicate that both parts of the estrogen-mediated signal transduction, the ligand and the receptor are active and produced by the tumour cells. This observation implicates potential use of targeted drugs for chondrosarcoma. Drugs that interfere with estrogen signaling are extensively applied for treating tumours, especially breast cancer. Either anti-estrogens or aromatase inhibitors could be the drugs considered for treating irressectable or metastatic chondrosarcoma.

40 Myosarcomatous Differentiation in Dedifferentiated Liposarcomas Does Not Increase the Risk of Metastatic Dissemination. Comparative Study between Conventional Dedifferentiated Liposarcomas (CDLPS), Dedifferentiated Liposarcomas with Divergent Myosarcomatous Differentiation (DLPS-MS), and Pure Leiomyosarcomas (LMS) of Retroperitoneal and Paratesticular Regions

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Background: CDLPS of the retroperitoneum and paratesticular regions are prone to recur locally but show a low metastatic rate. Conversely, distant metastases are frequent in pure LMS of same sites. The aim of this study was to assess the prognosis of DLPS-MS and to compare it to that of CDLPS and pure LMS, especially in terms of metastatic potential.

Design: 21 DLPS-MS, 66 CDLPS, and 28 LMS, all primaries, were reassessed clinically, morphologically, and immunohistochemically. The antibodies used included desmin, smooth muscle actin, h-caldesmon, myogenin, MDM2, CDK4, C-KIT, estrogen and progesterone receptors. Mean follow-up was 73months (range: 2-258months).

Results: In the DLPS-MS category, there were 12 males and 9 females aged from 40 to 88 yrs (median: 60 yrs). Sixteen tumors were in the retroperitoneum, 5 in the paratesticular area. Thirteen contained a rhabdomyosarcomatous (myogenin+) component, and 8 a leiomyosarcomatous (h-caldesmon+) component. Tumor size ranged from 5 to 35 cm (median: 16.5 cm), quite similar to that of LMS (2-20 cm, median: 12.5 cm, p=.03) and of CDLPS (7-45 cm, median: 20 cm, p non significant). All DLPS-MS and CDLPS were positive for MDM2 and CDK4, at least focally, whereas LMS were consistently negative for these markers. C-KIT, ER, and PR were negative in all cases. Overall and local recurrence-free survival rates were not significantly different between the DLPS-MS, CDLPS, and LMS subgroups, but the proportion of metastasis-free patients was significantly lower in the LMS category (36% at 5 years) as compared to CDLPS (80%, p<.0001) and DLPS-MS (71%, p=.05).

Conclusions: Patients with DLPS-MS and CDLPS of the retroperitoneum and paratesticular regions show comparable outcomes, but they have better metastasis-free survival rates as compared to those with LMS.

41 Lipoblastoma in Adolescents and Young Adults: Report of 6 Cases with Fluorescence In Situ Hybridization Analysis

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Background: Lipoblastoma is a rare benign tumor of infancy. Tumors can be localized (lipoblastoma) or diffuse (lipoblastomatosis) and show an admixture of mature and immature adipocytes (lipoblasts) in various stages of development. The majority of lipoblastomas possess rearrangements of the 8q12/*PLAG1* region. Another subset display polysomy for chromosome 8. Rare cases of lipoblastoma have been reported in older children and in adults, however without objective proof of the diagnosis such as molecular analysis, it is difficult to prove this diagnosis. We present six cases of lipoblastoma in adolescents and adults, with molecular analysis by fluorescent in situ hybridization (FISH).

Design: 6 cases previously diagnosed as lipoblastoma in adolescents (>12 yo) and adults were retrieved from our files. Clinical information and histological features were reviewed. Interphase FISH was performed on 4-µm formalin-fixed, paraffin embeded tissue sections using DNA α-satellite probes to chromosome 8 (CEP8-SO)

and chromosome 12 (CEP12-SG) (Vysis Inc., Downers Grove, IL), BAC probe RP11-22E14 and YAC probe 164h8 (flanking *PLAG1* breakpoint region), and BAC probes RP11-1064p9 (*MDM2*) / RP11-534n15 (*CDK4*) (Research Genetics, Huntsville, AL). Hybridization signals were visualized using an epifluorescence microscope (Leica DMRB, Wetzlar, Germany) equipped with a cooled CCD camera and image analysis software (QUIPS, Vysis, IL).

Results: There were 4 males and 2 females, 14 to 24 yo. The tumors involved the scrotum (2 cases), retroperitoneum, mediastinum, thigh and foot, and ranged in size from 9 - 25 cm. All cases showed prominent lobulation. Three tumors were mainly composed of mature adipose tissue, with only scattered lipoblasts. The 3 other cases were predominantly myxoid. FISH analysis showed rearrangements of the *PLAG1* region in 2 cases and polysomy for chromosome 8 in 3 other cases. None of the tumors had amplification of *MDM2/CDK4.*

Conclusions: Our data confirms that lipoblastoma occurs rarely in adolescents and young adults. Cytogenetics and/or FISH are powerful diagnostic tools that can be useful in differentiating lipoblastoma with an unusual clinical presentation from other diagnostic considerations such as atypical lipomatous tumor or myxoid liposarcoma.

42 Epstein-Barr Virus-Associated Smooth Muscle Tumors (EBVSMT): A Distinctive Smooth Muscle Tumor Associated with Multiple Infection Events

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Background: EBVSMT occurring in immunocompromised patients are rare tumors that have been incompletely characterized. We present the largest series to date with associated molecular analysis.

Design: Twenty-seven cases coded as EBVSMT were identified in 18 immunosuppressed patients from consultation files and assessed using in situ hybridization for EBV-encoded RNA (EBER) and immunohistochemistry (SMA, desmin, CD3 and CD20). To determine the number of "infection events" in multifocal cases, we assessed the long terminal repeat (LTR) region by real-time PCR using DNA from formalin-fixed, paraffin-embedded material. Presence of a deletion in the LMP1 gene (ΔLMP)was determined using PCR and relative amounts of EBV DNA by real-time PCR.

Results: Of 18 patients, 1 had SLE, 7 were HIV+ and 10 were renal transplant recipients. Tumors occurred in all ages (21-57 years, mean 40), more often in men (12M:6F) and were found in liver (4), oro/nasopharynx (4), lung (3), small bowel (2), extradural space (2), chest wall (2), spleen (2), abdominal wall, spinal cord, bladder, bone, adrenal gland, gall bladder, pleura and vocal cord (1 each). Tumors presented from 24-216 months (mean 80) after transplant/diagnosis. All tested tumors were EBER positive (25/25). Tumors expressed diffuse SMA (26/26), focal desmin (13/24) but not T- and B-cell markers (0/10) despite the common presence of intralesional lymphocytes. Tumor cells had 0-12 mit/10hpf (mean 3) and ranged in appearance from those resembling leiomyomas to leiomyosarcomas to primitive round cell tumors. Followup was available in 17/18 patients (1-105 months, mean 32) with 3 patients DWD, 4 patients ANED and 10 AWD. No tumors (0/6) had ΔLMP. Two patients with multifocal tumors had different numbers of LTR reflective of multiple infection events. The amount of EBV DNA/cell varied up to 30-fold between cases.

Conclusions: EBVSMT have a more varied appearance than conventional soft tissue smooth muscle tumors and often have intralesional lymphocytes. While tumor-associated morbidity is high, death was rarely directly attributable to tumor. In some patients, multifocality is attributable to separate infection events as opposed to one event with subsequent metastasis. Absence of ΔLMP suggests that wildtype LMP1 is sufficient for oncogenesis in this clinical setting. The amount of EBV DNA/cell varies greatly in EBVSMT.

43 Malignant Fibrous Histiocytoma of Bone Revisited: Immunhistochemical Analysis of 29 Cases

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Background: Malignant fibrous histiocytoma (MFH) of bone is a rare primary malignant neoplasm of bone characterized by pleomorphic spindled cells and storiform architecture. Recently, myoid differentiation has been shown to adversely affect prognosis in pleomorphic sarcomas of soft tissue. We present an immunohistochemical analysis of 28 cases of MFH of bone with a focus on myoid markers.

Design: Thirty-one cases coded as MFH of bone were retrieved from the consult files of one author (KKU) and from the University of Chicago Orthopedic database. Formalin-fixed paraffin-embedded tissue was assessed immunohistochemically for desmin, smooth muscle actin, muscle-specific actin, calponin, h-caldesmon, S100 protein and pancytokeratin.

Results: Following immunohistochemical analysis of 31 cases, two cases were reclassified as metastatic carcinoma and melanoma and 1 case was excluded due to insufficient material. Tumors were found in all ages (20 - 79, mean) and occurred equally in both sexes. Tumors were located in the femur (12), ilium (6), humerus (5), tibia (3), maxilla (3) and sternum (1). Tumors expressed smooth muscle actin (14/28), muscle-specific actin (13/28), calponin (8/28), S100 protein (2/28) and cytokeratin (2/28). All tumors were negative for desmin, h-caldesmon, and myogenin. Ten tumors were negative for all markers. Follow-up information was obtained for 26/28 patients (3 mos – 105 months, mean 35). Seventeen patients died with disease (mean survival 26.3 months), 2 were AWD(28 and 84 months) and 7 were ANED(33 to 104 months, mean 63.8, median). Three patients had recurrences and 10 had metastases to lung (8), bone (2), brain/spine (1) and lymph node (1). Of the patients with at least one myoid marker, 11/16 (69%) were dead with disease. Of the patients without any myoid markers, 6/12 (50%) were dead with disease.

Conclusions: Attempts at identifying a line of differentiation in MFH of bone have, unlike in those of soft tissue, been limited. Half of the cases in this study displayed myoid markers (calponin, smooth muscle actin, muscle specific actin), although desmin, h-caldesmon and myogenin were negative in all cases. These findings suggest that the neoplastic cell in MFH of bone may be myofibroblastic in origin. In this small study, there is no correlation between expression of myoid markers and prognosis.

44 Detection of Ewing and Synovial Sarcoma Fusion Transcripts Using Conventional and Real-Time PCR

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Background: Diagnosis of Ewing (ES) and synovial sarcomas (SS) can be problematic using histology and immunohistochemistry alone. Detection of characteristic fusion transcripts t(11;22) and t(21;22) in ES and t(X;18) in SS using RT-PCR can aid separation of these entities from each other and from other sarcomas. We have compared standard RT-PCR using gel electrophoresis with a rapid real-time approach.

Design: 102 frozen tissue samples of sarcomas diagnosed using morphological and immunohistochemical criteria were studied. These included 21 ES, 27 SS, 17 spindle cell sarcomas, 8 myxoid liposarcomas and 29 others. RNA extracted from each sample was reverse transcribed using random hexamers. The resulting cDNA samples were analysed by standard RT-PCR for t(11;22) (EWS/FLI-1), t(21;22) (EWS/ERG) and t(X;18) (SYT/SSX) followed by product analysis on 6% polyacrylamide gels. The same cDNA samples were studied using real-time analysis of EWS/FLI-1 and EWS/ERG (in a single reaction) and SYT/SSX, using dual-labelled probes. RT-PCR results using the two approaches were correlated with histological diagnosis.

Results: EWS/FLI-1 transcripts were detected in 15 cases (all ES; 71%) using conventional RT-PCR and in 14 of the same cases using real-time RT-PCR. Two of the real-time results were weakly positive. No EWS/ERG transcripts were detected. 27 SYT/SSX transcripts were detected in cases of SS (100%) in the same cases by both conventional and real-time RT-PCR. No EWS/FLI-1 or SYT/SSX transcripts were detected in non-ES or non-SS cases respectively, although non-reproducible late (Ct>35) real-time SYT/SSX signals were seen in three non-SS cases.

Conclusions: Conventional and real-time RT-PCR can detect sarcoma related fusion transcripts in a high proportion of ES and SS. Negative cases are likely to contain variant translocations. Identical results were achieved with conventional and real-time RT-PCR with SYT/SSX analysis, although there was one presumed false negative and two equivocal results in the real-time analysis with the EWS/FLI-1/ERG primers. This problem should be resolved by further optimization. This will permit routine use of real-time PCR with its advantages of speed, reduced contamination risk and potential for quantification. As false positives seem to be rare and these methods can be adapted to work with paraffin-embedded tissue, including decalcified samples, they offer a powerful aid to the diagnosis of these difficult tumours.

45 The Utility of Novel Fluorescence In-Situ Hybridization (FISH) Probes in the Diagnosis of Myxoid Soft Tissue Neoplasms

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Background: The diagnosis of myxoid soft tissue neoplasms is often challenging due to their overlapping histologic features. Molecular studies have identified several chromosomal translocations associated with subsets of myxoid sarcomas, including t(12;16)(*FUS-CHOP)* in myxoid liposarcomas (MLS), t(7;16)(*FUS-CREB3L2*) in low-grade fibromyxoid sarcomas (LGFMS) and t(9;22)(*EWS-CHN)* in extraskeletal myxoid chondrosarcomas (EMCS). Detecting such translocations in formalin-fixed, paraffin-embedded tissues by FISH would be convenient and useful. We report our experience with dual-color break-apart probes for *EWS*, *FUS*, and *CHOP* genes in a variety of myxoid neoplasms.

Design: Blocks from 37 cases were retrieved from our archives and a tissue microarray (TMA) was constructed to include myxomas (n=6), MLS (n=12), LGFMS (n=11), EMCS (n=5), myxoid MFH (n=2), and well-differentiated liposarcoma with myxoid change (n=1). The TMA sections were evaluated using break-apart probes spanning the genomic regions of *EWS* (22q12), *CHOP* (12q13) and *FUS* (16p11) genes (Vysis). All tumor nuclei in duplicate 1.5-mm diameter tissue cores were evaluated for the presence of fused (normal) or split (translocated) red-green signals.

Results: 11/12 (91.6%) MLS had chromosomal rearrangement of the *CHOP* gene (mean:69.5%+ cells/core; range:40-90%); 10/12 (83.3%) had changes in the *FUS* gene (mean:67.5%+ cells/core; range:40-95%). 1 MLS case (8%) had evidence of an *EWS* translocation (70% + cells). 4/11(36.3%) LGFMS had chromosomal rearrangement of the *FUS* gene (mean:71.3%+ cells/core; range:60-90%); with no changes in *EWS* or *CHOP*. 1/5 (20%) EMCS had evidence of an *EWS* translocation; none showed changes in *CHOP* or *FUS*. The remainder of the neoplasms studied were negative for changes involving *CHOP, FUS,* and *EWS.*

Conclusions: FISH using *FUS*, *CHOP* and *EWS* break-apart probes readily identifies the characteristic translocations in most MLS. There is a paucity of data on the frequency of *FUS* translocations in LGFMS; in this series, 36.3% showed a rearrangement in *FUS* by FISH. The low frequency of detectable *EWS* changes in this study of EMCS may be due to the small sample size or may reflect the relatively frequent occurrence of variant translocations in EMCS. Importantly, no non-translocation-associated myxoid tumor was positive with any probe, suggesting a definite role for FISH in this often-difficult differential diagnosis.

46 Subcutaneous Leiomyosarcoma in Children: A Clinicopathologic Study with Emphasis on Biologic Behavior

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Background: In all ages, approximately one-third of subcutaneous leiomyosarcomas (SQLMS) reportedly metastasize, especially to lung, and cause patient death. However, children seem to have a much better prognosis than adults, as demonstrated by individual cases and a small cohort from 1999. We wanted to explore the clinicopathologic and especially biologic behavior of a larger group of pediatric patients with SQLMS of the trunk and extremity to better understand the age-specific biologic behavior and potential treatment for these tumors.

Design: Cases coded as "subcutaneous leiomyosarcoma" in patients less than 20 years old were pulled from our files. Patient F/U and additional IHC were obtained.

Results: 18 SQLMS cases were included;11 cases were excluded based on dermal or non-trunk/extremity location(n=4), wrong or unknown patient age (n=5), or better diagnosis as angiomatoid (malignant) fibrous histiocytoma (n=1) or cellular fibrous histiocytoma (n=1). 11M:7F Ages ranged from 10 months to 19 (mean 11.3) years. All patients presented with mass (2 painful) for 2-4 months duration. Size ranged from 2.0-5.8 (mean 3.4) cm. All cases were circumscribed and spindled, two had epithelioid features, with eosinophilic cytoplasm and perinuclear clearing. Many also involved fascia. Additional features included giant cells (n=2), pericytoid vascular pattern, especially at periphery of lesion (n=9), pleomorphism (n=3), lymphocytic response (n=3), and focal myxoid change (n=2). Mitoses ranged from 1 to >20/10 hpf; necrosis was absent in all but one case. All cases, except three high grade (3/3), were graded as 1/3 by FNCLCC. All cases studied were strongly positive for SMA and calponin and all except 5 for either desmin and/or caldesmon. Additionally 3 cases had focal CK18; CD117, S100 protein, CD34, ER/PR, CD68, and pankeratins were all negative. Treatment included surgical excision alone. Follow-up on grade 1/3 so far reveals no evidence for recurrence or metastasis, range 1-13 (mean 8.7) years follow-up.

Conclusions: SQLMS in patients less than 20 years old have good prognosis. Surgical treatment alone for grade 1-2/3 seems to be effective in these patients. Pericytoid vascular pattern is common in these SQLMS of younger patients and should not be confused with benign tumors.

47 Palmar-Plantar Fibromatosis in Children and Preadolescents: A Clinicopathologic Study of 56 Cases with Long-Term Follow-Up

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Background: Palmar-plantar fibromatosis (PPF) is very rare in juveniles and has not been adequately evaluated in this age group. This retrospective study, based on AFIP material, represents the first large series of PPF in children and preadolescents.

Design: Fifty-six cases of PPF in patients ≤12 yrs of age, diagnosed over 28.5 yrs, were examined in detail and compared to > 600 cases of PPF from other age groups.

Results: Our series included 19 males and 37 females, 2-12 yrs old at first surgical intervention (median: 9 yrs). The patients typically presented with solitary, lobular or multilobular masses, 0.5-2.5 cm in size. All but 2 of the initial lesions involved the plantar aspect of the feet, typically in the region of the arch. Only 2 patients presented with palmar disease. The tumors were usually painless, except when pressure was applied. Six patients had a history of trauma. One patient presented with bilateral disease involving both feet, and 10 additional patients subsequently developed PPF in another extremity or had other findings linked to the disease complex. A family history (FH) was available for 23 patients, and 11 had a (+) FH and 2 had a history suspicious for disease in close relatives. The preoperative duration for the initial lesions was 1 mo-6 yrs; 1 patient had evidence of disease since birth. Histologically, all tumors involved (and were typically confined to) aponeurosis. The tumors commonly formed discontinuous, moderately cellular, nodular masses composed of spindled cells with intervening collagen. In contrast with the palmar lesions, plantar examples characteristically formed larger and more well-defined nodular masses with a paucicellular, "umbilicated" center. Mitotic counts for 80 tumor specimens ranged from 0-31 mitotic figures (MF)/25 wide-field HPFs (mean: 3.45 MF/ 25 WHPFs). Eight tumors had ≥10 MF/25 WHPFs. All patients were initially managed by local excision, and in almost all cases, tumor involved the tissue edge. Thirty-two of 36 patients (89%) with follow-up of 4 mos-31 yrs (median: 14 yrs), had one (n=16) or more (n=16) local recurrence of their fibromatosis.

Conclusions: In the juvenile population, PPF has a very high recurrence rate when managed by local excision. A (+) FH is often present, consistent with an autosomal dominant pattern of inheritance. Some patients develop multifocal disease and knuckle pads, and there appears to be an increased incidence of seizures, and possibly keloid formation and clinodactyly.

48 Perivascular Epithelioid Cell Neoplasms (PEComas) of Soft Tissue and Gynecologic Origin: A Clinicopathologic Study of 24 Cases

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Background: PEComas, occasionally associated with the tuberous sclerosis complex, are defined by the presence of perivascular epithelioid cells, which co-express muscle and melanocytic markers. This family includes angiomyolipoma (AML), "sugar" tumor of the lung, lymphangioleiomyomatosis (LAM), and very rare tumors in other locations. Since non-AML/ non-LAM PEComas are extremely rare and their natural history and prognostic features undefined, we present our experience with 24 PEComas of soft tissue and the gynecologic tract, the largest series to date.

Design: All PEComas exclusive of AML and LAM were retrieved from consultation files. Immunohistochemistry for CK, S100 protein, SMA, desmin, vimentin, HMB45, Melan-A, MITF, TFE3, c-kit and CD34 was performed. Follow-up information was obtained. Fisher's exact test was performed.

Results: Median age was 49 years (range 15-97 years); there was a marked female predominance (21F, 3M). Locations included mesentery (N=5), uterus (N=5), somatic soft tissue (N=4), pelvis (N=4), cervix/vagina (N=3), falciform ligament, broad ligament, and skin (1 each). The tumors ranged from 1.6-29 cm (median 7.2 cm). Tumors were epithelioid (N=10), spindled (N=4) and mixed (N=10). Multinucleated giant cells were present in 18 cases. High nuclear grade was noted in 10 cases, high cellularity in 7 cases, necrosis in 8 cases, and vascular invasion in 3. Mitotic activity was 0-50 MF/ 50 HPF (median 0 MF/50 HPF) with atypical MF in 6 cases. IHC results: SMA (19/23), desmin (8/20), HMB45 (19/22), Melan-A (12/16), MITF (9/16), S100 protein (5/22), CK (3/21), vimentin (10/12), TFE3 (5/14), c-kit (1/17), CD34 (0/7). Follow-up (N=18, mean 26 months, range 5-84 months): 13 NED, 2 AW local recurrence, 3 AW metastases (liver, lung, bone), 1 DOD. LR and/or MET was strongly associated with size > 7 cm (p= 0.009), necrosis (p= 0.005), MR> 1/50 HPF (p= 0.0005), but not nuclear grade, cellularity, atypical MF, vascular invasion or epithelioid morphology.

Conclusions: PEComas of soft tissue and gynecologic origin may be benign or malignant. Malignancy was correlated with large size in the face of any level of mitotic activity, and necrosis. Clinically benign PEComas may show marked nuclear pleomorphism alone (symplastic). Occasional PEComas express unusual markers, such as S100 protein, desmin and rarely CK. The role of TFE3 in PEComas should be further studied.

49 Incidence of *NF1* Deletion and t(X;18) in Synovial Sarcoma and Malignant Peripheral Nerve Sheath Tumor

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Background: Malignant peripheral nerve sheath tumor (MPNST) may show significant histologic and immunohistochemical overlap with synovial sarcoma (SS), particularly the monophasic variant. Because of this, many have sought to utilize molecular testing as an adjunct to diagnosis for these difficult cases. Although t(X;18) is a well-established finding in SS, the literature bears conflicting accounts regarding the presence of this alteration in MPNST. Deletions of *NF1*(17q11.2) are frequently detected in both NF1-associated and sporadic MPNST, however the incidence of this alteration in SS is unknown.

Design: Tissue microarrays were constructed to include 17 SS (mono- and biphasic variants), 13 MPNST, and 10 other spindle cell tumors (fibrosarcoma (5), hemangiopericytoma (2), schwannoma (1), epithelioid sarcoma (1), sarcoma not otherwise specified (1)). 25 tumors (11 SS, 7 MPNST, and 7 other spindle cell tumors) had previously determined t(X;18) status by reverse transcriptase-polymerase chain reaction (RT-PCR) performed on fresh tissue samples in our clinical molecular pathology laboratory. Fluorescence in situ hybridization (FISH) was utilized on these tissue microarray sections to determine *NF1* dosage. A dual color break-apart FISH probe cocktail (Vysis, Downers Grove, IL) containing two large DNA probes flanking the SYT (18q11.2) breakpoint region served to validate t(X;18) status.

Results: 94% (16/17) of SSs and 31% (4/13) of MPNSTs had demonstrable t(X;18). This alteration was not detected in any of the other spindle cell tumors. There was 100% correlation between RT-PCR and FISH assays for t(X;18) determination in samples where both methods were employed. Deletions of *NF1* were encountered in 33% (4/12) of MPNSTs but in none of the SSs. One case deemed a fibrosarcoma by histology was found to harbor a *NF1* deletion. None of the samples tested were found to have concomitant *NF1* deletion and t(X;18).

Conclusions: Our findings suggest that both SS and a subset of MPNST may harbor t(X;18), and that RT-PCR and FISH are equally suitable techniques for routine detection of this alteration. Deletion of *NF1* is also encountered in MPNST, though mutually exclusive of t(X;18), and is not a feature of SS.

50 MLH-1 and MSH-2 Immunoreactivity and Microsatellite Instability: A Comparison of Clear Cell Sarcoma and Metastatic Melanoma

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Background: The genetics of soft tissue sarcomas may be conceptually divided into two categories: those with tumor specific translocations, and those with complex karyotypic defects. The latter may represent a manifestation of defective DNA repair mechanisms. Clear cell sarcoma (CCS) of soft tissue shares features of melanoma, but, unlike melanoma, CCS shows a unique t(12;22) translocation. Therefore, comparisons between CCS and melanoma may serve as a useful model system to study cell transformation in sarcomas. Microsatellite instability (MSI) has been observed in melanoma but not CCS. Furthermore, defects in the mismatch repair enzymes MLH-1 and MSH-2 have been implicated in MSI in several malignancies, including melanoma metastases. The expressions of MLH-1 and MSH-2 and their relationship to MSI are not well characterized in CCS.

Design: The purpose of this study was to determine whether any difference in MSI between CCS and melanoma correlates with absence of nuclear MLH-1 and/or MSH-2 immunoreactivity. We identified 11 cases of CCS by histology, immunohistochemistry and the presence of the characteristic t(12;22) translocation. The CCS cases were compared to a group of 11 metastatic melanomas (MM) for the presence of hMLH-1 and hMSH-2 by standard immunohistochemical techniques. Cases with ≥5% of cells showing specific, moderate to strong, nuclear staining were considered positive and were tabulated based on the fraction of cells staining. MSI, as

well as loss of heterozygosity (LOH) were determined using a fluorescence-based PCR for five microsatellite markers (BAT25, BAT26, D5S346, D17S250, D2S123).
Results: All 11 cases of CCS (100%) showed specific nuclear staining for both MLH-1 and MSH-2. None of the CCS cases showed MSI, although loss of heterozygosity (LOH) was observed in 1 case. In contrast, MLH-1 and MSH-2 immunoreactivity were present in 8/11 (73%) of MM, a difference that was only nearly statistically significant ($p<0.10$). MSI, defined as instability in at least 2/5 markers, was observed in 3/11 (27%) of the MM but did not correlate with the absence of MLH-1 and MSH-2 immunoreactivity.
Conclusions: CCS and melanoma are phenotypically similar in many respects. However, MSI and loss of MLH-1 and MSH-2 in melanoma, but not in CCS, suggest a difference in karyotype complexity between these tumors. A definite relationship between MSI and the absence or defect of MLH-1 and MSH-2 in melanoma remains to be determined.

51 Utility of CD117 Immunoreactivity in Differentiating Metastatic Melanoma from Clear Cell Sarcoma

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Background: Clear cell sarcoma (CCS) is a malignant soft tissue tumor with melanocytic differentiation. Unlike melanoma, CCS demonstrates a t(12;22) translocation. When the diagnosis is uncertain, such as in a deep soft tissue or visceral mass without a known melanoma primary, molecular methods may help distinguish between CCS and melanoma. Previous studies have shown that CD117 immunoreactivity is not typically seen in CCS. This finding is intriguing because CD117 is expressed in a subset of melanomas and, furthermore, expression may inversely correlate with melanoma progression. CD117 immunoreactivity may be useful in separating melanoma from clear cell sarcoma.
Design: We identified 20 tumors in our surgical pathology files that were diagnosed as CCS or in which CCS was strongly considered because of (1) melanocytic differentiation (2) deep soft tissue or visceral location and (3) no known primary melanoma. These were tested for the presence of the t(12;22) translocation by RT-PCR performed on paraffin embedded tissue, followed by sequencing of PCR products. Cases with a t(12;22) translocation were immunostained with an antibody to CD117 using standard methods and compared to 14 cases of metastatic melanoma. Moderate to strong cytoplasmic/membrane staining in ≥5% of cells was considered positive.
Results: Of the 20 tumors, 11 demonstrated the t(12;22) translocation. In each case, RT-PCR and sequencing confirmed the presence of the fusion transcript between exon 7 of the EWS gene and codon 65 of the ATF-1 gene. The remainder either did not demonstrate any of the previously reported fusion transcripts or were noninformative and were excluded from further study. No control cases of metastatic melanoma demonstrated the t(12;22) translocation. None of the 11 cases of CCS (0%) showed membrane or cytoplasmic staining for CD117. Conversely, 12 of 17 (71%) metastatic melanomas were positive for CD117, a difference that was significant ($p<0.001$). Interestingly, all three cases in which CCS was initially considered as a diagnosis, but which lacked t(11;22) were positive for CD117.
Conclusions: RT-PCR, performed on paraffin embedded tissue, is a useful, rapid tool for identifying the presence of the t(12;22) translocation thereby, confirming the diagnosis of CCS. CD117 immunoreactivity may prove useful in the differential diagnosis of deep soft tissue or visceral lesions with melanocytic differentiation, as positive staining excludes CCS but is compatible with metastatic melanoma.

52 Loss of *EXT* Expression Causes Downregulation of IHh Signaling in Hereditary and Solitary Osteochondromas

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Background: Loss of *EXT1* or *EXT2* is implicated in the development of osteochondromas in context of the hereditary disorder, Multiple Osteochondromas (MO). Both genes were shown to act as tumor suppressor genes for MO. Mutations in one of the *EXT* genes in solitary osteochondromas are extremely rare, however loss of heterozygosity (LOH) of the *EXT1* locus can be found. The EXT1/2 complex is involved in the biosynthesis of heparan sulphate proteoglycans, which are involved in IHh/PTHrP signaling. We previously showed the signaling molecules downstream in this pathway (PTHrP, PTHR1 and Bcl-2) to be mostly absent in both hereditary and solitary osteochondromas. Expression of *EXT1* and *EXT2* and the relation to their direct downstream effectors in the IHh/PTHrP pathway, *IHh*, *Ptc*, *Smo* and *Gli2*, is investigated in both solitary and hereditary osteochondromas. Both *Ptc* and *Gli2* can be used as a direct read-out system for IHh signaling, since they are transcribed upon activation of IHh signaling.
Design: cDNA was made from RNA isolated from hereditary (n=3) and solitary (n=4) osteochondromas and post-natal epiphyseal growth plates (n=4). Primers were designed for *EXT1*, *EXT2*, *IHh*, *Ptc*, *Smo*, *Gli2* and five housekeeping genes. Quantitative RT PCR reactions were performed with Sybr Green. To allow comparison between osteochondromas and growth plates, the results were normalized based on the mean expression of the housekeeping genes in the study group.
Results: *EXT1* was downregulated in solitary osteochondromas and osteochondromas from MO patients with an *EXT1* mutation, compared to normal growth plate ($p<0.001$). Interestingly, *EXT2* was downregulated in all hereditary osteochondromas ($p=0.002$) but not in the solitary ones ($p=0.54$). This is in parallel to the LOH that is only found at the *EXT1* locus in solitary osteochondromas. In both solitary and hereditary osteochondromas, expression of *Ptc* and *Gli2* was lower than in growth plates ($p=0.05$ and 0.03 respectively). *IHh* and *Smo* were not differentially expressed ($p=0.73$ and 0.95 respectively) suggesting constitutive gene expression.
Conclusions: Despite the absence of somatic *EXT* mutations, we show *EXT1* but not *EXT2* to be downregulated in solitary osteochondromas. Moreover, this is the first direct proof that low or absent *EXT* expression due to mutations and/or LOH resulting in downregulation of IHh/PTHrP signaling causes both hereditary and solitary osteochondromas.

53 Progression of Secondary Peripheral Chondrosarcoma Is Characterized by Downregulation of Oxidative Phosphrylation Genes

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Background: Osteochondroma can occur solitary or in a hereditary syndrome, Multiple Osteochondromas. Loss of both copies of either *EXT1* or *EXT2* in hereditary osteochondromas implicates involvement of *EXT*-related pathways. On genomic level, malignant transformation of osteochondroma towards peripheral chondrosarcoma is a multistep process. Genome wide expression analysis was performed on a well-documented series of peripheral cartilaginous tumors and normal growth plates to elucidate pathways involved in development of osteochondromas, malignant transformation to and progression of peripheral chondrosarcomas.
Design: RNA isolated from osteochondromas (n=6), secondary peripheral chondrosarcomas (n=14) and normal growth plates (n=4), was hybridized against a reference panel (RNA mix of 15 tumor cell lines) using tyramid signal amplification, on a custom made cDNA microarray containing 9000 clones enriched for cartilage specific genes and printed in duplicate. For data analysis we used several biostatistics including hierarchical clustering, Significance Analysis of Microarray and an application for finding entire significant pathways. Sequencing, quantitative RT PCR and/or immunohistochemistry verified a subset of differentially expressed genes.
Results: Cluster analysis revealed great similarity in gene expression between osteochondromas and epiphyseal growth plates, which is in parallel with the morphological resemblance of osteochondromas and growth plates. None of the analyses revealed significant differently expressed genes between osteochondromas and grade I chondrosarcomas, which corroborate the difficulty to histologically distinguish low-grade chondrosarcoma from its benign precursor. Progression towards high-grade tumors was characterized by downregulation of cartilage specific extracellular matrix genes (ECM), like *COL9* and *COL2*, and upregulation of others such as *COL4* and *LUM*. In addition three genes encoding proteins of the oxidative phosphorylation complexes I and III were significantly downregulated in high-grade tumors, compared to low-grade tumors.
Conclusions: Progression towards high-grade peripheral chondrosarcoma is characterized by downregulation of the oxidative phosphorylation genes. Progression also demonstrates downregulation of cartilage specific ECM, which parallels loss of the hyaline cartilage matrix seen at microscopy.

54 EXT-Related Pathways Are Not Involved in Pathogenesis of Dysplasia Epiphysealis Hemimelica and Metachondromatosis

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Background: Dysplasia Epiphysealis Hemimelica (DEH, Trevor's disease) and metachondromatosis (MC) are considered in the clinicoradiological and histological differential diagnosis of solitary and hereditary (Multiple Osteochondromas, MO) osteochondromas. DEH is a rare developmental disorder with cartilaginous overgrowth of a portion of one or more epiphyses. MC is a rare autosomal dominant disorder exhibiting both enchondromas and osteochondromas that, radiographically, in contrast to MO, point toward the joints and may regress spontaneously. Loss of *EXT* is involved in the development of MO. *EXT* displays its function in the biosynthesis of heparan sulphate proteoglycans, which are involved in growth signaling pathways in the human growth plate. We have previously demonstrated that in hereditary and solitary osteochondromas *EXT*-related pathways are downregulated.
Design: Fixed paraffin embedded material from 10 DEH cases and 2 MC cases were used for immunohistochemistry using antibodies against Syndecan-2, CD44, CD44v3, HS-chains, PTHrP, PTH-R, Bcl-2, and p21. Results were compared with previous data from osteochondromas. RNA isolated from frozen material from one DEH and one MC case were included in genome wide cDNA expression array analysis and quantitative PCR analysis of *EXT* pathways and compared with solitary and hereditary osteochondromas.
Results: At the protein level *EXT* downstream targets are expressed in most DEH and MC, while these are downregulated in osteochondromas (table 1).
Table 1 Immunohistochemical results

	Osteochondroma	DEH	osteochondroma vs DEH	MC	osteochondroma vs MC
antigen	pos	pos	p-value	pos	p-value
Syndecan-2	15/21	8/8	0.15	2/2	1
CD44	3/23	0/8	0.55	0/2	1
CD44v3	19/20	8/9	0.53	2/2	1
HS-chain	4/22	1/10	1	0/2	1
PTHrP	37/65	9/9	0.011	2/2	0.51
PTH-R	12/19	6/7	0.38	2/2	0.53
Bcl2	3/59	4/6	0.001	0/1	1
p21	5/20	3/7	0.63	2/2	0.09

cDNA expression analysis DEH and MC clustered completely separate from solitary and MO osteochondromas. *EXT1* was expressed in DEH and MC, while lost in osteochondromas.
Conclusions: Despite histological similarities to solitary and hereditary osteochondroma, lesions of DEH and MC are separate entities as confirmed by their different cDNA and protein expression profiles. More specifically, *EXT* downstream targets, that are downregulated in osteochondroma, are expressed in DEH and MC, suggesting that *EXT* signaling is not disturbed in the latter two disorders.

55 Desmoplastic Fibroma of Bone: An Immunohistochemical Study on 13 Cases Including β-Catenin Expression and Mutational Analysis for β-Catenin

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Background: Desmoplastic fibroma of bone is a rare primary bone tumor resembling desmoid type fibromatosis of soft tissue. The aims of this study are to investigate the immunohistochemical profile and the involvement of the β-catenin pathway in desmoplastic fibroma as in its more common soft tissue counterpart, namely the desmoid type fibromatosis.

Design: Immunohistochemistry was performed on thirteen cases of desmoplastic fibroma for muscle-specific markers, estrogen- and progesterone receptors, CD117, β-catenin and the potential downstream targets of β-catenin namely Cyclin-D1. On all thirteen cases DNA sequencing was performed for the detection of activating β-catenin gene (CTNNB1) mutations.

Results: There was no immunoreactivity of CD117, estrogen and progesterone receptors. Seven cases were immunoreactive for one or more muscle-specific markers. In 6 cases there was over expression of β-catenin in the cytoplasm with in one of these cases also accumulation of β-catenin in the nucleus. In 6 cases, in which DNA sequencing was successful, no β-catenin mutations were detected.

Conclusions: The epidemiological, histological and immunohistochemical findings in desmoplastic fibroma are suggestive of desmoplastic fibroma being the bony counterpart of the more common desmoid type fibromatosis of soft tissue. However, the β-catenin pathway does not seem to have the same essential role in the tumorigenesis of desmoplastic fibroma as it has in desmoid type fibromatosis.

56 Extensively Cytokeratin-Positive Schwannomas: A Little-Recognized Diagnostic Pitfall

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Background: Most schwannomas can be confidently diagnosed by routine microscopy. Those with unusual features (e.g. hypercellularity, pseudoglandular change) may require immunohistochemistry, however. The overwhelming majority of schwannomas express S100 protein and often GFAP , but not cytokeratins, actins, or desmin. A very small number of schwannomas reportedly show focal cytokeratin expression. We report 7 schwannomas with near-uniform cytokeratin positivity, which resulted in significant diagnostic difficulty for the referring pathologist.

Design: Seven histologically typical, S100 protein-positive cases, coded as showing cytokeratin positivity, were retrieved from our consultation archives. When available, additional sections were immunostained for pancytokeratins (CK), high molecular weight cytokeratins (HMWCK), glial fibrillary acidic protein (GFAP), neurofilaments (NF), and desmin using steam heat-induced epitope retrieval and the Dako Envision system. Additionally, sections from 23 other schwannomas were retrieved from our archives and immunostained for CK. Cases were scored as negative, "1+" (1-25% positive), "2+" (26%-75% positive) or "3+" (>75% positive).

Results: The 7 strongly cytokeratin-positive tumors occurred principally in older adults(median 55 years, range 17-77 years) of either sex (4 F, 3 M). The tumors ranged from 3.2 – 10 cm and occurred in the posterior mediastinum (2 cases), sacrum, neck, psoas muscle, chest wall, and retroperitoneum (1 case each). All showed 3+ (5 cases) or 2+ (2 cases) positivity for CK. 3+ expression of vimentin (5/5) and GFAP (3/3) was also present. No case expressed HMWCK (0/3), desmin (0/6) or NF (0/3). Rare CK-positive cells were identified in 7/23 (30%) ordinary schwannomas.

Conclusions: Near-uniform CK-positivity in schwannomas is previously unreported and extremely rare. Careful attention to the stereotypical histologic features of these tumors (e.g., Verocay bodies, thick walled vessels) and to their otherwise typical immunophenotype (S100 protein, GFAP and vimentin-positive) should prevent misdiagnosis of these lesions as carcinoma or as one of the acknowledged sarcomas with cytokeratin expression (e.g. synovial sarcoma). CK-positivity is more frequent in schwannomas than has previously been thought, possibly because of epitope retrieval; this may reflect true anomalous expression, or possibly cross-reactivity of CK antibodies with GFAP, as has been shown in gliomas.

57 Soft Tissue Perineurioma: Analysis of 81 Cases Including Examples with Atypical Histologic Features

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Background: Perineuriomas are uncommon benign peripheral nerve sheath tumors that include soft tissue, sclerosing and intraneural variants. Fewer than 50 soft tissue perineuriomas have been reported to date, and the clinical significance of atypical histologic features is unknown.

Design: To characterize these tumors further, 81 soft tissue perineuriomas were retrieved from the authors' consult files between 1994 and 2003. H&E sections were re-examined, immunohistochemistry was performed, and clinical details were obtained from referring physicians.

Results: 43 patients were female and 38 male (mean age 46 years; range 10-79). Tumor size ranged from 0.3-20 cm (mean 4.1). Most patients presented with a painless mass. The tumors arose in a wide anatomic distribution: 36 lower limb, 19 upper limb, 19 trunk, and 7 head & neck. 41 tumors were situated in subcutis, 25 in deep soft tissue, and 10 were limited to the dermis. Nearly all cases were grossly well-circumscribed; 12 showed focal microscopically infiltrative margins. Most tumors had a storiform and focally whorled growth pattern; 17 exhibited fascicular areas. 38 were hypocellular, 15 were markedly hypercellular, and 7 showed alternating zones of hypo- and hypercellularity. Stroma was usually collagenous but in 17 tumors was predominantly myxoid, and in 16 was mixed collagenous and myxoid. Mitoses ranged from 0-13 per 30 HPF (mean 1); 53 had no mitoses. Based on worrisome cytologic or architectural features, 14 cases were classified as atypical perineuriomas: 12 contained scattered pleomorphic cells, 1 showed an abrupt transition from typical morphology to a markedly hypercellular, fascicular area with cytologic atypia, and 1 exhibited diffuse infiltration of skeletal muscle. All tumors were reactive for EMA; 50/78 (64%) expressed CD34, 22/76 (29%) claudin-1, 16/77 (21%) SMA, and 4/81 (5%) S-100. All cases were negative for GFAP, NFP, and desmin. Follow-up was available for 42 patients (mean 39 months; range 6-146). 31% of tumors were marginally excised, 19% had positive margins, and 50% were widely excised. Two tumors recurred locally (one of which was atypical): one recurred 10 years following primary excision; and one recurred twice, 5 and 9 years following excision. No tumor metastasized.

Conclusions: Soft tissue perineuriomas behave in a benign fashion and rarely recur. Atypical histologic features (including scattered pleomorphic cells and infiltrative margins) seem to have no clinical significance and appear to be akin to those seen in ancient schwannoma or atypical neurofibroma.

58 Signaling Molecules in Primary and Metastatic Synovial Sarcoma; a Tissue Microarray Study

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Background: Synovial sarcoma (SS) is a malignant soft tissue tumor that can show morphological and immunophenotypic evidence of epithelial differentiation. Signaling molecules involved in the development of epithelial malignancies including the wnt-ß-catenin pathway, E-cadherin and the epidermal growth factor receptor (EGFR) have been implicated in SS and their expression reportedly has prognostic relevance. However, it is not known whether the expression of these markers differs between primary and metastatic tumors. Recent evidence has also shown that ß-catenin regulates cyclin D1 in carcinoma and some soft tissue neoplasms. Interestingly, SYT-SSX (the product of the t(X;18) translocation in SS) may also regulate cyclin D1 in SS.

Design: Eighty-two tumors initially identified as SS based on a combination of morphology, immunophenotype and ultrastructure were incorporated into a tissue microarray. The presence of the t(X;18) translocation was tested by fluorescence in situ hybridization (FISH) of the array. Cases without the translocation or with equivocal FISH results were excluded. Standard immunohistochemical methods using antibodies to EGFR, ß-catenin, cyclin D1 and E-cadherin were used. Cases in which ≥5% of cells stained were considered positive and were tabulated based on the fraction of cells staining and the location of staining.

Results: Fifty-nine tumors demonstrated the t(X;18) translocation and were further studied. With one exception, immunoreactivity for E-cadherin (12/59, 20%) was identified only in the epithelioid component of biphasic tumors. EGFR (27/59, 46%), ß-catenin (51/59, 86%) and cyclin D1(39/59, 66%) were identified, at least focally, in both components of biphasic tumors and in monophasic tumors. There was no significant difference in immunoreactivity for any of the markers between a primary and the corresponding metastasis or recurrence. A significant association was noted between the presence of nuclear ß-catenin and cyclin D1 ($p<0.025$).

Conclusions: In general, the presence of immunoreactivity in a primary tumor predicts the presence in the metastasis/recurrence suggesting that the expression patterns of these markers may be established early during tumorigenesis, prior to metastasis. The correlation between nuclear ß-catenin expression and cyclin D1 expression suggests a role for beta catenin induction of cyclin D1 accumulation in SS.

59 Novel Gene ANKS1 Expression in Gastrointestinal Stromal Tumors

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Background: Precise classification of gastrointestinal stromal tumors (GISTs) is important because GISTs respond well to targeted therapy with imatinib mesylate (Gleevac). Recent studies have shown that in a minority of cases there is an absence of KIT (CD117) immunostaining in tumors that have clinicopathologic features of GIST. Thus, additional GIST-specific markers would be useful in classifying challenging cases.

Design: We examined a previously published set of 42,000 spot cDNA gene expression arrays for 26 GISTs and identified strong expression of a novel gene, *ANKS1*, encoding an ankyrin (ANK)-repeat protein of unknown function, which appeared to be specific for GISTs when compared to the other mesenchymal tumors. Digoxigenin-labeled sense and antisense RNA probes for *ANKS1* were generated by *in vitro* transcription using T7 tagged *ANKS1* specific primers. In situ hybridization (ISH) was performed on a tissue microarray of 155 soft tissue tumors, including 26 GISTs.

Results: Greater than 90% of GISTs analyzed by cDNA microarray showed strong expression of *ANKS1* that was independent of mutation status. Expression for *ANKS1* was verified on tissue microarray and showed 14/26 (54%) GISTs demonstrating strong, diffuse *ANKS1* signal on ISH. A subset of neoplasms in the differential diagnosis of GISTs also showed ISH reactivity, including: 4/30 (13%) LMS; 2/40 (5%) MFH; 2/12 (17%) MPNST; 2/10 (20%) SFT; 2/9 (22%) synovial sarcoma; and 3/9 (33%) rhabdomyosarcomas.

Conclusions: *ANKS1*, a gene of unknown function, is expressed in greater than 90% of GISTs on cDNA microarray analysis. We showed that *ANKS1* is expressed strongly in 54% of GISTs tested with ISH. The gene encodes an ANK-repeat protein that may be associated with an important signal transduction mechanism in GISTs as well as other soft tissue tumors

60 Plexiform Fibrohistiocytic Tumor - A Clinicopathologic Study of 38 Cases

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Background: Plexiform fibrohistiocytic tumor (PFT) is a rare fibrohistiocytic lesion affecting predominantly young female patients. Although the previous studies have emphasized the tendency of tumor to behave in an indolent fashion, the regional and systemic metastatic potential of this tumor has been recognized. Short clinical follow-up and/or small numbers of patients in these studies have limited the assessment of long-term biologic behavior of this tumor.

Design: We have identified 38 cases of PFT. Clinical history and follow-up were obtained by reviewing the patients medical records and/or through the correspondence with referring clinicians and patients. Pathologic features of the tumors were collected through the careful review of H&E stained slides by two independent reviewers.

Results: We have obtained a clinical follow-up (1 year or longer; mean length of follow-up 6.3 years) on 27 patients. In this time period, 8 patients (29.6%) developed tumor recurrence, 4 patients (14.8%) developed distant metastases and 3 patients (11.1%) died of the disease. Four of the patients in whom tumor recurred locally had an inadequate initial tumor resection (focally positive margins or possibly positive, close margins). Information about the extent and the completeness of the initial surgical procedure could not be determined for the other 4 patients. No correlation could be established between histologic features of tumors and their biologic behavior.

Conclusions: This study has confirmed the trend observed in previous studies. PFT is a tumor of young, female patients with predilection for the extremities. Although this tumor predominately behaves in an indolent manner, extended follow up shows that it can lead to systemic metastases and death in a small group of patients. Biologic behavior of the tumor can not be reliably predicted from its morphologic features. Tumor recurrence is more likely to occur in cases where inadequate surgical resection was performed. Development of distant metastases is the only factor that correlates with poor survival.

61 STAT3 Activation in Ewing Sarcoma Family of Tumors

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Background: Signal transducer and activator of transcription 3 (STAT3) is an oncoprotein that has emerged as a promising molecular target for cancer therapy. Constitutive activation of STAT3 has been detected in diverse human cancer cell lines and tissues. Nevertheless, whether STAT3 is activated in the Ewing Sarcoma Family of Tumors (ESFT) has not been determined. In this study, we assessed STAT3 activation in ESFT using an antibody against tyrosine-phosphorylated STAT3 (pSTAT3) and correlated these findings with biological and clinical characteristics.

Design: Twenty three formalin-fixed paraffin-embedded pretreatment tumors with morphologic and immunophenotypic features of ESFT were utilized to construct a duplicate-core tissue microarray. Fluorescence in situ hybridization (FISH) was performed using a dual-color break-apart probe cocktail (Vysis, Downers Grove, IL) flanking the EWS-R1 breakpoint region (22q12). Tissue microarray sections were surveyed for STAT3 activation, defined as nuclear staining with pSTAT3 in more than 20% of tumor cells. Ki-67 immunostaining was utilized to assess the proliferation rate. Terminal dUDP nick end-labeling (TUNEL) assay was performed to assess the apoptotic rate.

Results: Demonstrable rearrangements involving the EWS gene locus, as evidenced by red and green signal separation using the EWS break-apart probe set, were identified in all 23 tested cases. Immunostaining for pSTAT3 was identified in 15/23 (65%) cases. STAT3 activation was associated with a high (>5/1000cells) apoptotic rate (75% vs. 8%; p=0.022 Fisher's exact test). No correlation between STAT3 activation and proliferation rate was identified. Clinical data was available on 19 patients with a median follow-up of 4.5 years (range, 0.6-23.1 years). Patients were 6 females (32%) and 13 males (68%) with a median age of 13.7 years (range, 2.6-19.9 years). Eight patients had metastatic disease. The 5-year overall survival and event-free survival for pSTAT3-positive cases was 69.2% and 42.2% compared to 33.3% and 16.7% for pSTAT3-negative cases, respectively (p=0.47 and p=0.36, Fisher's exact test).

Conclusions: STAT3 activation is identified in a subset of ESFT and may be associated with a more favorable clinical outcome.

62 True Leiomyosarcoma of Gastrointestinal tracT. A Clinicopathologic and Molecular Genetic Study of 43 Cases

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Background: A study of true gastrointestinal (GI) leiomyosarcomas LMSs was recently reported, however little is known about their frequency, clinicopathologic and genetic features. These tumors, previously often diagnosed as LMSs, may be confused with malignant gastrointestinal stromal tumors (GISTs) characterized by specific morphologic, immunophenotypic and genetic features including KIT expression and presence of KIT or PDGFRA activating mutations.

Design: In search of true GI LMSs, 3500 primary GI mesenchymal tumors retrieved from AFIP files were evaluated histologically, immunohistochemically and for GIST-specific KIT exon 9, 11, 13 and 17 and PDGFRA exon 12 and 18 activating mutations.

Results: 43 primary GI mesenchymal tumors showing morphologic features characteristic of peripheral soft tissue LMSs were identified. All these tumors expressed smooth muscle actin and 60% of them were positive for desmin. CD34 expression was seen in 12% of analyzed cases but no KIT positivity was detected. No GIST-specific mutations in KIT and PDGFRA were found in 31 analyzed tumors. There were 24 males and 19 females (M/F ratio 1.3:1). The patient age ranged from 24 to 88 years but more than 80% of patients were 50 years or older. The majority (85%) of these true GI LMSs involved intestines, however a few were esophageal (n=3) and omental (n=2). Only 1 true GI LMS was gastric in the cohort of 2000 cases. Most true GI LMSs presented as polypoid or non-polypoid intraluminal masses, however a few tumors were externally attached to the intestinal wall. Tumor size ranged from 2 to 20cm. Histologically, all had pure spindle cell morphology with well-differentiated smooth muscle cells. Most true GI LMSs were high-grade tumors with at least focal pleomorphism and high mitotic activity (>20/50HPF). Follow-up data were available in 24 cases. Although more than 50% of patients died of the disease with an average survival of 31 months, prognosis of anorectal tumors appeared to be better. One patient treated with tyrosine kinase inhibitor, Gleevec, showed clinical progression of the disease during treatment.

Conclusions: True GI LMSs represent a specific entity with characteristic clinicopathologic features. These tumors have to be separated from KIT negative GISTs.

63 Amplifications of *CDK*4 and *MDM*2 Genes in Epithelioid Sarcomas Detected by Array-Based Comparative Genomic Hybridization and Fluorescence *In Situ* Hybridization

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Background: Epithelioid sarcomas (ES) is a distinctive, aggressive soft tissue tumor typically presenting as a subcutaneous or deep dermal mass in the distal extremities of young adults. Previous studies using conventional cytogenetic approaches have described abnormalities in several chromosomal regions. However, the details of molecular genetic defects underlying the development of ES remain elusive. Recently developed array-based comparative genomic hybridization (A-CGH) has many advantages over traditional methods for investigation of genetic aberrations in neoplasms, allowing high-throughput analysis of copy number changes at high resolution on a genome-wide scale. This facilitates the identification of novel genetic aberrations that could be used for diagnostic and prognostic markers or as therapeutic targets.

Design: We employed A-CGH to characterize the genetic alterations in two well-characterized ES cases with available frozen tissue. The frequency and type of genetic aberrations were first assessed on a low-density array that contains approximately 300 targets throughout the genome including oncogenes and tumor suppressor genes (Vysis). The study was then extended using Roswell Park Cancer Institute 6000 bacterial artificial chromosome (BAC) high-density array. Fluorescence *in situ* hybridization (FISH) was performed using BAC-labeled probes to validate selected A-CGH results.

Results: A-CGH showed that the total number of amplifications was markedly greater than that of deletions in both studied cases. While high-level amplification of 12q13-15 regions, including *CDK*4 (12q13-14) and *MDM*2 (12q14-15) genes, was seen as a dominant feature in one case, the second case showed only moderate amplification of *CDK*4. These genetic alterations were revealed by both the low- and high-density arrays. Furthermore, FISH studies using probes specific for *CDK*4 or *MDM*2 confirmed the A-CGH findings.

Conclusions: The results demonstrate that A-CGH and FISH validation can be effective tools for identification of new potential diagnostic and prognostic markers in soft tissue neoplasms such as ES. Amplification of *CDK*4 and *MDM*2 genes may play a pivotal role in ES pathogenesis. This study will be extended to a larger number of ES cases to establish whether there is a relationship between amplifications of *CDK*4 and the tumorgenesis of ES.

64 Usefulness and Limitations of Core Biopsy in the Diagnosis and Management of Musculoskeletal Tumors and Pseudotumors. A Multidisciplinary Approach in 136 Cases

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Background: Ultrasound-guided needle biopsy is a safe and efficient method for the histologic diagnosis, increasingly used in the initial approach for superficial and deep musculoskeletal tumors. However, its reliability in daily practice for therapeutic decisions still has not reached a generalized consensus.

Design: During a 5-year period (1999-2003), 136 patients with musculoskeletal tumors were studied following the protocol established for Soft Tissue & Bone Tumors by the oncology Comitteee group of our Institution. The tumors were located in the extremities and thoraco-abdominal wall. All patients were biopsied under ultrasound guidance using a 18 BioPince® gauge needle in an attempt to know the histology of the tumor prior to surgery. Tissue cores were immersed in cold saline serum and immediately submitted to the Pathology Lab. Cytologic (imprints and serum centrifugation) and histologic material was disposable for the diagnosis in all cases.

Results: There was a female predominance (80F/56M) with an age average of 55,8 years (range 2-88). Lesions were located in the soft tissues in 127 cases, in nine cases the tumor was primarily osseous with soft tissue component. The sites involved were: lower extremity (42), abdominal wall (20), upper extremity (20), dorsal (17), thoracic wall (16), upper (5) and lower (11) girdles, and head & neck (5). The diagnostic categories were summarized as follows: benign/reactive tumor (51), sarcoma (30), metastatic carcinoma (25), normal tissue NOS (19), lymphoma (6), and others (5). Surgery was not performed in 57 cases (19 benign tumors, 15 carcinomas, 11 normal tissue NOS, 7 sarcomas, and 5 lymphomas). Core biopsy was followed by surgery in 79 cases. Biopsy/surgery diagnostic discordances were seen in 13 cases (16,4%) (11 lipomas and 2 well differentiated liposarcomas were labelled as fatty tissue NOS). Incomplete diagnosis of sarcoma or carcinoma was done in 10 cases (12,6%). In 2 cases (2,5%) the method was not helpful.

Conclusions: Fatty tumors were the major source of discrepancies, but in most cases its importance was irrelevant. Chronic inflammatory disorders, carcinomas, and lymphomas may also appear as soft tissue/bone tumor masses. Overall, the diagnosis of sarcoma was accurate and allowed an appropriate approach to surgery. Only in a few cases the method could not provide useful information for treatment.

65 Immunohistochemical and Electron Microscopical Evidence That the Non-Osteoblastic Components in Osteoid Osteoma and Osteoblastoma Are Similar and Distinctive

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Background: In spite of their histological similarity, osteoid osteoma (OO) and osteoblastoma (OBL) have traditionally been regarded as somewhat distinct entities on the basis of their different size, symptoms and skeletal distribution. Apart from the identification of a nerve supply in OO, there is little published work on the nature of the non-osteoblastic element of OO and OBL.

Design: From the archives of our institutions, we retrieved typical clinico-radiologico-pathologic examples of OO (ten cases), spinal OBL (ten cases), and non-spinal OBL (ten cases) for immunohistochemistry (IHC) with the following primary antibodies: S100, neurofilament, PGP9.5, neuron specific enolase, epithelial membrane antigen (EMA), broad spectrum cytokeratins, desmin, caldesmon, smooth muscle actin (SMA) and CD 34. We performed electron microscopy (EM) on selected OOs and OBLs.

Results: With IHC, we highlighted the already well recognised neural supply characteristic of OO, but also identified a similar neural supply in spinal and non-spinal OBL. Furthermore, in the intertrabecular space in both OO and OBL, we identified sheets of small, spindle cells expressing EMA and a variable number of isolated, large, desmin positive, SMA/caldesmon negative cells with smudged/degenerate nuclei. In selected OOs and OBLs, EM of the large, desmin positive cells showed striking nuclear membrane complexity, abundant and often mis-shaped mitochondria and dilated rough endoplasmic reticulum. There was no EM evidence to suggest rhabdomyoblastic differentiation.

Conclusions: Our findings show that, in the intertrabecular space, OO and OBL share common IHC and EM features that are distinctive and appear to be intrinsic to the tumorous process. In such richly innervated tumors, the EMA expression by the abundant, small intertrabecular spindle cells suggests perineurial/meningothelial differentiation. The specific differentiation pathway of the desmin-expressing cells is not clear.

We present new evidence that OO and OBL together represent a single tumor entity and, furthermore, should not be considered as purely osteoblastic tumors. Rather, these tumors may be neoplasms with divergent differentiation or, alternatively, may represent neural/perineural proliferations with secondary, florid osteoblastic reaction.

66 Primary Versus Radiation-Associated Craniofacial Osteosarcoma: Biologic and Clinicopathologic Comparison

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Background: The majority of radiation-associated sarcomas (60%) are osteosarcomas; 10% occur in the craniofacial region, especially the jaws. Compared with primary craniofacial osteosarcoma, radiation-associated osteosarcoma (RAOS) tends to be larger, higher grade, metastasize more frequently, and show poorer 5-year disease-free survival. Surgical excision is the mainstay of curative therapy and the role for chemotherapy remains unproven. To better understand the biology of craniofacial RAOS, we studied clinicopathologic, immunohistochemical, and p53 mutational features, and compared them to those in primary craniofacial osteosarcoma.

Design: Clinical data was obtained by retrospective review of patient records. Archival paraffin-embedded tissues from 22 patients with head and neck osteosarcoma were reviewed and evaluated for expression of p53, pRb and MIB1 by routine immunohistochemical techniques. p53 mutation analysis was performed by separately amplifying and sequencing exons 5-8.

Results: Six (27%) patients had prior radiation with a mean 16 years prior to diagnosis (range 9-24). Two of these had hereditary retinoblastoma. All RAOS were high grade compared to only 8 (50%) primary osteosarcomas. Three RAOS were fibroblastic and 3 osteoblastic. Only 1 primary osteosarcoma was fibroblastic, 9 osteoblastic and 6 chondroblastic. RAOS had higher p53 expression (50% vs.19% were p53+) and higher proliferative activity (4 of 6 with >50% MIB1 staining vs. 0 of 16). pRb expression was similar in both. Two RAOS and only 1 primary osteosarcoma had p53 mutation. Two RAOS patients died of disease, 3 were alive with unresectable disease, and 1 was lost to follow-up. By contrast, only 1 of 16 patients with primary osteosarcoma died of disease, 2 were alive with disease, 12 were disease-free, and 1 was lost to follow-up.

Conclusions: Craniofacial RAOS is more aggressive than primary craniofacial osteosarcoma. RAOS tends to be higher grade, have fibroblastic histology, overexpress p53, have mutated p53, and have higher proliferative activity compared to primary craniofacial osteosarcoma. Evaluation of these feautures may be useful in predicting prognosis and determining which patients might benefit from adjuvant or neoadjuvant chemotherapy.

67 Irradiation of Myxoid and Round Cell Liposarcoma Induces Growth Arrest and Lipogenic Maturation

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Background: There is a general belief that radiation treatment is of limited value in low grade sarcomas; however, there are a few reports of MLS illustrating a remarkable response to radiation therapy. Moreover, multicentric MLS and multiple soft tissue metastases may be difficult to treat solely with surgery. The aim of this study was to investigate the clinical and morphological effects of radiotherapy in the treatment of MLS/RCLS.

Design: 29 primary and metastatic MLS/RCLS in 15 patients were treated with radiotherapy. 23 tumors were surgically removed after preoperative radiation therapy (34-46 Gy), while 6 tumors were treated solely with radiotherapy (44 - 60 Gy). Preoperative diagnosis was made using FNA or core needle biopsy in most cases. 13 tumors were genetically characterized before and after radiation therapy. Tumor size was measured by CT or MRI before and after radiotherapy in 26 tumors. 23 tumors were surgically removed after radiation therapy and morphologic response evaluated.

Results: Four patients had multicentric / metastastic tumor disease; one of these patients developed 36 soft tissue MLC before expiring at 99 months. 12 of 13 tumors demonstrated a FUS/CHOP translocation before and after radiation therapy. 75% of irradiated tumors showed a median reduction in tumor volume of 54% with non-systematic changes in MRI-enhancement pattern; in 4 cases, the tumor was no longer detectable after radiation therapy. The most striking morphological changes observed after radiation therapy included lipogenic maturation, paucicellularity, hyalinization and an extremely low proliferative index. No major differences in response to radiation therapy were seen between the 9 patients with purely MLC vs. the 6 patients with mixed MLS/RCLS. Follow-up showed that 11 patients were continuously disease free (6 - 77 mos.); 3 patients had a tumor-related death (39 - 99 mos.); and one patient is alive with disease at 62 mos.

Conclusions: Radiation therapy of MLS/RCLS induces growth arrest and lipogenic maturation. Radiation therapy is a viable treatment option in patients with multiple unresectable MLC/RCLS. In some cases, it can lead to significant tumor reduction thereby facilitating surgical resection.

68 Gastrointestinal Stromal Tumors (GISTs) of Stomach in Children and Young Adults – A Study of 46 Cases

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Background: GISTs are uncommon in children and their clinicopathologic profile has not been well established.

Design: Forty-six cases of GISTs in the age group 0-21 years were retrieved from AFIP files and evaluated histologically, immunohistochemically, and for KIT mutations in exons 9,11,13 and 17 and PDGFRA mutations in exons 12 and 18. Follow-up was obtained.

Results: There were 23 females and 23 males ranging from 5 to 21 years, the youngest being a 5 year-old boy and an 8 year-old girl. All but one patient under the age of 16 were females. The most common presentation was insidious, sometimes symptomatic GI-bleeding and anemia. The tumors measured 1.5-24 cm (median 5.6cm). The majority of tumors with specified location were in the antrum (17 of 22); 5 were in the body. One patient had evidence of Carney triad, also having a pulmonary chondroma. Histologically, 32 tumors had predominantly epithelioid and 14 spindled morphology. Mitotic activity varied from 0-65/50 HPFs (median 6/50 HPFs). KIT-positivity was found in 22/23 cases, CD34 in 20/24; there was no SMA, desmin or S100-positivity (0/24). Only one of 12 patients studied had KIT exon 11 mutation; none had PDGFRA mutations; the mutation positive patient was 19 yrs old. Six patients died of liver metastases with median survival of 16 years (range 5.5-35.5 yrs); three of these had a tumor >5cm and mitoses >5/50HPFs. Four patients were alive with liver metastases with median follow-up 14 yrs, and sixteen patients were alive with no evidence for disease with a median follow-up of 17 years (range 7-40 yrs).

Conclusions: Gastric GISTs in children and young adults are typically epithelioid tumors that most often occur in the antrum. They are very rare in the first decade. Most tumors are KIT- positive, but compared with GISTs of adults, KIT and PDGFRA mutations are rare. The tumor behavior varies but is somewhat unpredictable. The course of disease is often protracted with liver metastases sometimes developing 10 or more years after primary surgery.

69 Epidermal Growth Factor Receptor (EGFR) Pathway in Osteosarcoma (OS): An Immunohistochemical Study

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Background: In OS, finding markers of prognostic or therapeutic significance continues to be of importance. The EGFR pathway is of interest due to the recent application of tyrosine kinase inhibitors. The phosphatidylinositol-3 kinase (PI3K/AKT) pathway, activated downstream of EGFR, is involved in cell survival, and has been implicated in resistance to chemotherapy. This pathway and the mitogen-activated protein kinase (MAPK) pathway are involved in cell proliferation. Although EGFR expression has been shown in OS, its role in OS remains to be further analyzed. The aim of this study is to examine this pathway in OS by immunohistochemistry.

Design: 96 samples of primary OS (69 patients) including multiple subtypes were reviewed. 27 patients had matched pre (PR) and post (PT) chemotherapy specimens (n=54). A tissue microarray was constructed and immunostaining was performed using antibodies to EGFR, MAPK, p-AKT, and Ki-67. Expression was scored as an index (intensity x %) with index ≥100 considered positive. Ki-67 proliferation index was recorded as % positive tumor nuclei. Correlation and regression analysis was performed including comparison of pre/post treatment data.

Results: In 69 patients, EGFR staining was seen in 22/48(45%) of PR OS and 13/48(27%) of PT OS. p-AKT staining was seen in 28/46(61%) of PR OS and 30/47(63%) of PT OS. MAPK staining was seen in 9/47(19%) of PR OS and 3/47(6%) of PT OS. Ki-67 proliferation rates were 10.5% for PR OS and 5.7% for PT OS. Significant differences between pre and post means for EGFR (p=0.003) and MAPK (p<0.001) were found. Spearman correlation analysis found significant positive correlation in PR specimens between EGFR and p-AKT (p<0.001), EGFR and Ki-67 (p=0.004) and p-AKT and Ki-67 (p=0.004). Regression analysis indicated that EGFR in PT specimens was a significant predictor for Ki-67 and p-AKT. This association was not significant after adjusting for p-AKT (p=0.06) in multivariate analysis, suggesting a mediating effect of p-AKT on EGFR in relation to Ki-67. MAPK was not significantly involved in this association.

Conclusions: EGFR expression is higher in PR specimens and a significant correlation was identified between EGFR, p-AKT and Ki-67. In PT specimens, EGFR was a predictor for Ki-67 proliferation index and p-AKT. This suggests that the EGFR pathway may be involved in the pathogenesis of OS through P13K rather than MAPK, resulting in increased cell survival and proliferation. Further study of this pathway in OS is needed to explore possible targets for pharmacologic therapy.

70 Spindle Cell Liposarcoma/Atypical Lipomatous Tumor: A Clinicopathologic Study of 120 Cases

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Background: The spindle cell variant of liposarcoma (LPS)/atypical lipomatous tumor (ALT) has not been studied extensively. The aim of this study is to better define its morphologic spectrum and biologic behavior.

Design: One hundred and twenty cases of pure spindle cell LPS/ALT were retrieved from the consult files of one of the authors. Hematoxylin-and-eosin stained sections were available for review in all cases. Clinical and follow-up information was obtained from the referring pathologists and clinicians.

Results: Seventy-nine patients were male and 41 were female. Patients' age ranged from 6 to 82 years (median 52). In the majority of the cases, tumors were located in the subcutaneous tissue of surgically amenable sites: trunk (28 cases), upper extremities (23), lower extremities (18), head and neck (24), hand (8) and feet (4). Other sites of involvement included vulva (6), mesentery (1), pharynx/larynx (2), pleura (1), mediastinum (1), hip (2), scrotum (1) and perineum (1). Clinically, most patients presented with a slowly growing, painless mass or swelling. Treatment was usually by surgery alone. Seventy-three percent of the patients were alive without evidence of disease (median follow-up 37 months) and 19% of the patients showed at least one local/regional recurrence. One patient developed dedifferentiated LPS and died of disease. Microscopic examination revealed a wide spectrum of histologic appearances. Typically, adipocytic and spindle cell components were intermixed in varying proportions. The adipocytic component showed variation in adipocyte size and shape, scattered atypical, hyperchromatic stromal cells and occasional univacuolated or multivacuolated lipoblasts. The spindle cell component was composed of fascicles of uniform ovoid to elongated small cells, with bland nuclei, inconspicuous nucleoli, and moderate amounts of palely eosinophilic cytoplasm. Stromal collagen and hyalinization were variably present. Mitoses were rare (median 1/30 hpf) and necrosis was absent. Tumor margins were ill-defined with invasion into surrounding tissues.

Conclusions: Spindle cell LPS/ALT is a disease of adults, with male predilection. It typically arises in somatic soft tissue with 87.5% of the cases involving trunk, extremities, and head and neck. Recognition of the range of histologic appearances and identification of the neoplastic adipocytic component are key features for the diagnosis of this entity. Due to approximately 20% risk of local recurrence, treatment should include surgical resection with wide margins.

71 Diffuse Malignant Mesothelioma of the Peritoneum: A Clinicopathologic Study of 35 Cases

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Background: Diffuse malignant mesotheliomas (DMMs) are uncommon tumors and a majority of cases occur in the pleura, followed by the peritoneum, with the relative frequency being 10 to 1. The tumor growth is characterized by peritoneal seeding and ascites formation, eventually resulting in patient's death due to tumor encasement and invasion of bowel and intractable ascites. Recent trials of multimodality therapy have resulted in long-term survival of selected patients.

Design: 35 patients were uniformly treated by cytoreductive surgery (CRS) and intraperitoneal hyperthermic perfusion (IPHP). Histologic subtypes were classified to epithelial, sarcomatoid and biphasic type, and epithelial type was further divided into solid (S) and tubulopapillary (TP) by predominant pattern. Immunohistochemistry stains were performed for calretinin, WT-1, pCEA, Ber-EP4, EGFR, p16, MMP-2 and MMP-9, and scored to 0 (negative), +1 (<25%), +2 (25-50%), +3 (50-75%) and +4 (75-100%). Statistic correlation was evaluated for age, sex, completeness of cytoreduction (CC), histologic subtype, mitotic count (MC), necrosis, nuclear grade (NG), and biological markers with overall and progression free survivals (OS and PFS).

Results: There were 15 male and 20 female patients. Age ranged from 24-73 years (median 52). 25 out of 35 patients were optimally cytoreduced. Follow-up ranged from 0.4-86 months (median 19). There were 32 epithelial (19 S, 16 TP) and 3 biphasic types with no cases of sarcomatoid type. There were 3 cases for NG1, 19 for NG2, and 13 for NG3. MC ranged from 0 to 160/50 HPFs (mean 14.1). Necrosis was present in 11 cases. Immunohistochemistry results are as follows:

Score	EGFR	p16	MMP-2	MMP-9
0	2	14	0	5
+1	1	11	2	9
+2	3	6	3	8
+3	7	2	7	8
+4	22	2	23	5

Calretinin and WT-1 were expressed in all cases to a variable degree, and pCEA and Ber-EP4 were negative in all tumors. NG (grade1/2 vs 3) and MC (≥5 vs 5>) were significantly correlated with OS (p=0.02, 0.01) while CC was correlated with both OS (p=0.05) and PFS (p=0.03). No biological markers were of prognostic value.

Conclusions: NG, MC and CC can be useful prognostic factors in patients treated by CRS and IPHP. Our results of expression of EGFR, MMP-2 and MMP-9 and loss/reduced p16 in DMMs confirmed the results of previous studies and suggest their role in tumor pathogenesis and kinetics.

72 Dedifferentiated Leiomyosarcoma; Clinicopathologic Analysis of 8 Cases

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Background: Leiomyosarcomas exhibiting dedifferentiation analagous to that occurring in liposarcoma or chondrosarcoma have been very rarely described and remain incompletely characterized.

Design: Eight cases of dedifferentiated leiomyosarcoma (0.6%) were identified from among a total of 1394 leiomyosarcomas in the author's consultation files through 2003. Pathologic features, immunohistochemical profile and available clinical data were evaluated to characterize these tumors further.

Results: Seven patients were female and one was male. Age distribution ranged from 62 to 89 years (median 78.5 years). The anatomic sites involved were retroperitoneum (3), uterus (1), arm (2), knee (1) and paratesticular region (1).Tumor size ranged from 2cm to 28cm (median 13cm). The gross appearance varied from pale and whorled to tan and lobulated. Necrosis was present and extensive in 5 cases. Tumor margin varied from well-circumscribed to infiltrative. Histologically, all tumors were characterized by a biphasic appearance. All exhibited areas typical of a low grade smooth muscle neoplasm with fascicles of eosinophilic spindle cells with cigar-shaped nuclei and variable mitotic rate. In all cases there was an abrupt transition to foci with a much more high grade pleomorphic (anaplastic) appearance in which myogenic features were not obvious. Immunohistochemically, within the areas exhibiting smooth muscle differentiation on H&E stain, all tumors were diffusely positive for at least one marker indicative of myogenic differentiation (SMA 7/7; Desmin 8/8; Caldesmon 2/2). However, these markers were uniformly absent within the dedifferentiated foci. Follow-up information was available in 4 cases; One patient with a retroperitoneal tumor died of liver metastases 12 months after resection of the primary tumor; One patient died 8 months after upper limb amputation; One patient had evidence of lung metastases 6 months following preoperative radiotherapy and resection of a knee mass. One patient who presented with a paratesticular mass had no evidence of recurrent disease or metastases at 5 months following resection with negative margins, chemotherapy and radiotherapy.

Conclusions: We describe 8 cases of dedifferentiated leiomyosarcoma. The phenomenon of dedifferentiation in leiomyosarcomas is uncommon. Based on this series such tumors may involve a range of sites and appear to occur most frequently in elderly females. The limited follow-up information available so far supports an aggressive clinical course, similar to that in dedifferentiated chondrosarcoma.

73 Novel USP6 Fusion Oncogenes with *TRAP150, ZNF9*, Osteomodulin, and *COL1A1* in Aneurysmal Bone Cyst Variant Translocations

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Background: Aneurysmal bone cyst (ABC) is a locally recurrent bone tumor that often features chromosome 17p13 rearrangements. One of the ABC 17p13 rearrangements – t(16;17)(q22;p13) – was recently shown to create the *CDH11-USP6* fusion gene in which the *USP6* oncogene is overexpressed through juxtaposition with the *CDH11* promoter. Four alternative ABC translocations involving 17p13 are described and each one of them results in a novel *USP6* fusion oncogene.

Design: Four ABC with variant chromosome 17p13 rearrangements were studied for the presence of novel fusion genes using 5' rapid amplification of cDNA ends - reverse transcriptase polymerase chain reaction (5'RACE RT-PCR). Genomic breakpoints were confirmed by fluorescence in situ hybridization (FISH) with bacterial artificial chromosomes (BACs).

Results: Four ABCs with chromosomal translocations t(1;17)(p34;p13), t(3;17)(q21;p13), t(9;17)(q22;p13) and t(17;17)(q12;p13) were identified. 5'RACE RT-PCR and sequencing analyses revealed *USP6* fusions with genes that are frequently expressed in mesenchymal/osteoblast cells, including *TRAP150* on 1p34*, ZNF9* on 3q21*, Osteomodulin* on 9q22, *and COL1A1* on 17q12, respectively. The structure of the novel fusion genes was akin to *CDH11-USP6*, with the *USP6* coding sequences juxtaposed to the promoter regions in each of the four translocation partners. FISH analyses confirmed the genomic breakpoint in each instance.

Conclusions: Our studies suggest that the heterogeneous 17p13 genomic rearrangements in ABC have a consistent functional consequence: namely, *USP6* transcription upregulation due to its juxtaposition to active promoters in the mesenchymal/osteoblast cell context. The four novel fusion oncogenes reported here, and the *CDH11-USP6* oncogene reported previously, establish an oncogene model in which USP6 overexpression may be sufficient for transforming activity in mesenchymal cells.

74 Cluster Analysis of Immunohistochemical Profiles in Malignant Peripheral Nerve Sheath Tumor (MPNST), Ewing Sarcoma (EWS) and Synovial Sarcoma (SS) Segregates Distinct Tumor Subpopulations

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Background: Because of overlapping morphologic and immunohistochemical features, MPNST, EWS and SS can be difficult to distinguish, especially in small biopsies. In order to further characterize their immunohistochemical profiles, we evaluated these tumors with a panel of markers and hierarchical cluster analysis.

Design: Tissue microarray slides constructed from paraffin embedded specimens of 23 MPNST, 27 EWS, and 23 SS were stained with a panel of 19 antibodies regarded as useful in the differential diagnosis. Intensity of staining was scored (0, 1 and 2) with attention to distribution and cellular location. For CD99, staining was categorized as generalized or membranous (CD99M). Euclidean hierarchical cluster analysis was performed using Gene Miner software.

Results: The following percentages of tumors (MPNST, EWS, SS, respectively) stained intensely for the markers used: S100 (39, 44, 39), NGFR (30, 11, 13), CD99 (48, 93, 65), CD99M (0, 74, 26), EMA (4, 7, 70), AE1/AE3 (9, 22, 65), CK7 (0, 0, 57), CK19 (0, 4, 48), CD56 (4, 0, 22), BCL-2 (9, 26, 43), Vimentin (91, 74, 96), B-Catenin (13, 44, 43), MMMP2 (5, 0, 11). No significant staining for CD57, Chromogranin, Synaptophysin, Calponin or ALK was detected in any tumor. Cluster analysis revealed six main profiles. The first two groups were all SS, 1 being CK+, EMA+ (n=14) including 6 CD99M+ tumors; the other being CK-, EMA+, CD56+, BCL-2+ (n=5). The third group was mostly CD99M+ EWS (n=23), but included 1 CD99M+, BCL-2+, CD56+ SS which lacked immunoreactivity for epithelial markers. The fourth group, characterized by S100 and BCL-2, comprised 3 EMA+ SS and 1 EMA- MPNST. The fifth and sixth groups contained mostly MPNST, 1 group being S100+, NGFR+ (n=8) and included 1 EWS and 1 SS; the other group S100-, NGFR+/- (n=13). A small group (n=3) of CD99+, CD99M- EWS clustered separately as an outlier.

Conclusions: Hierarchical cluster analysis derived 6 groupings of tumors within the 3 morphologic diagnoses, with very little overlap between groups. This could be helpful clinically, when selecting stains for difficult to classify tumors. However, these data also highlight pitfalls and limitations of immunohistochemistry with these tumors: 1) CD99M is not entirely specific for EWS, since over 25% of SS can be positive. 2) Multiple epithelial markers including EMA, pan-CK and CK subsets may be required for SS. 3) There is no reliable marker for MPNST, due to similar positivity rates for S100 in EWS and SS.

75 Heterotopic Mesenteric Ossification: A Distinctive Intraabdominal Pseudotumor Simulating Extraskeletal Osteosarcoma

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Background: Heterotopic mesenteric ossification is a rare intraabdominal bone producing pseudosarcoma with fewer than 10 reported cases in the literature. We present our experience with 5 additional cases, all of which were referred to us with a diagnostic consideration of extraskeletal osteosarcoma.

Design: Five intraabdominal lesions coded as "heterotopic mesenteric ossification", "ossifying pseudotumor" or "reactive myofibroblastic proliferation with ossification" were retrieved from our consultation archives. Clinical follow-up information was obtained.

Results: The cases occurred exclusively in males, with a mean patient age of 44 years (range 22-65 years). The tumors occurred in the mesentery (N=3), omentum (N=1) or both (N=1) and were preceded by significant abdominal surgery (3 cases) or trauma (1 case) in all but 1 case. Four patients presented with bowel obstruction and 1 with intraabdominal sepsis. The tumors were difficult to precisely measure; the mean size of the resection specimens was 13.4 cm (range, 5-20 cm). Grossly the tumors resembled fat necrosis, and often cut with a gritty sensation. Microscopically, all lesions demonstrated an exuberant, reactive (myo) fibroblastic proliferation resembling nodular fasciitis, with extensive hemorrhage and fat necrosis. All tumors produced abundant bone and osteoid, often "lace-like", and 2 contained cartilage. The proliferating (myo) fibroblasts, osteoblasts and chondroblasts were mitotically active but cytologically bland. Follow-up (4 cases, mean 47.3 months, range 5-120 months) showed 3 patients to be alive without disease, and 1 to be dead of unrelated causes.

Conclusions: Heterotopic mesenteric ossification is a distinctive intraabdominal ossifying pseudotumor that typically occurs in males, almost always after surgery or abdominal trauma. This clinical history, the presence of clearly reactive zones resembling ordinary nodular fasciitis, and the absence of nuclear atypicality, necrosis and atypical mitotic figures should allow the distinction of HMO from its most important morphologic mimic, extraskeletal osteosarcoma.

76 Dual-Color, Break-Apart Fluorescence In Situ Hybridization for *EWS* Gene Rearrangement Distinguishes Clear Cell Sarcoma of Soft Parts from Malignant Melanoma

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Background: Clear cell sarcoma of soft tissues (malignant melanoma of soft parts) is a soft tissue sarcoma with melanocytic differentiation that typically occurs in the tendons and aponeuroses of young adults. As can be demostrated by cytogenetics and RT-PCR, between 70% to over 90% of CCS have a t(12;22) translocation, fusing the *EWS* and *ATF1* genes on chromosomes 22q12 and 12q13, respectively. Identification of this translocation distinguishes CCS from histologic mimics, most importantly conventional malignant melanoma. We report our experience with a new commercially available, dual-color, break-apart FISH probe, which allows detection of the presence of *EWS* (22q12) gene rearrangments in formalin-fixed, paraffin-embedded tissues.

Design: A tissue microarray was constructed from histologically and immunophenotypically well-characterized cases of clear cell sarcoma (n=6) and malignant melanoma (n=32). An array section was analyzed with a 22q12 dual-color, break-apart probe (Vysis), which spans the known common breakpoints in the *EWS* gene on chromosome 22 (introns 7 through 10). Signals from tumor cell nuclei were counted under a fluorescent microscope and the presence of red-green break-apart signals was recorded.

Results: Of the CCS cases, 50% (3/6) showed evidence of an *EWS* gene rearrangement with a mean of 66.7% positive cells per sample (range: 60%-70%). All cases of malignant melanoma (32) showed virtually absent break-apart signals in the *EWS* gene (less than 4% cells per case).

Conclusions: FISH detects *EWS* gene rearrangement in a substantial proportion of clear cell sarcomas, with excellent specificity. Importantly, *EWS* FISH is negative in malignant melanoma, a clinically dissimilar tumor which may closely mimic clear cell sarcoma histologically and immunohistochemically. As this probe can be utilized in routinely processed tissue, FISH provides an excellent alternative to RT-PCR in cases where fresh tissue is not available.

77 Initial Experience with a Dual-Color, Break-Apart Fluorescence In Situ Hybridization (FISH) Assay for Detection of *SYT* Gene Rearrangements in Synovial Sarcoma

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Background: Synovial sarcomas (SS) are mesenchymal neoplasms that predominantly arise in the paraarticular regions of adolescents and young adults. The majority of SS harbor a t(X;18) translocation involving the *SYT* gene (18q11) and its partners *SSX1* or *SSX2* genes on chromosome X. Documenting the presence of these gene rearrangements provides important ancillary information for distinguishing SS from its histologic mimics, most prominently malignant peripheral nerve sheath tumor (MPNST). We report our initial experience with a new commercially available dual-color, break-apart probe, which allows detection of *SYT* gene rearrangements in routine formalin-fixed, paraffin-embedded tissues.

Design: A tissue microarray was constructed from 27 histologically and immunophenotypically characterized cases of SS (19 monophasic, 8 biphasic), spindled MPNST (n=14), gastrointestinal stromal tumor (n=13), leiomyosarcoma (n=1), malignant hemangiopericytoma (n=1), and mesothelioma (n=1). Array section was analyzed by FISH using a dual-color, break-apart *SYT* probe (Vysis). Signals from tumor cell nuclei in each tissue core were enumerated, negative showing apposed red-green signals and positive showing split signals.

Results: All 27 (100%) SS cases demonstrated the presence of a chromosomal break in the region of the *SYT* gene at 18q11, with a mean of 81% positive cells per sample (range: 62-94%). The 30 tumors in the differential diagnosis of SS showed a near absence of break-apart signals with a mean of 2% positive cells per sample (range 0-4%).

Conclusions: Our experience demonstrates that FISH offers a rapid, sensitive and specific approach to resolving the differential diagnosis of synovial sarcoma in difficult cases. The dual-color, break-apart FISH probe applied to routine paraffin-embedded tissue enables detection of a *SYT* gene rearrangements and constitutes a convenient alternative to RT-PCR analysis in cases where frozen tissue is unavailable.

78 Ezrin Expression in Gastrointestinal Stromal Tumors (GIST): A Comparison with Tumors of Smooth Muscle and Nerve Sheath Origin

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Background: Ezrin is a member of the ezrin-radixin-moesin (ERM) family which regulates linkage between membrane proteins and actin. Ezrin plays a role in microvillous formation and cell polarity in many epithelial cells. Ezrin expression has been correlated with growth and tumor invasion in several types of carcinomas, and has been correlated with metastasis. In osteosarcoma and rhabdomyosarcoma, high expression of ezrin correlates with ability to metastatize. Based on apparent ezrin staining in Cajal cells in the myenteric plexus of the small bowel, we analyzed the expression of ezrin in gastrointestinal stromal tumors (GIST) and compared it to a group of tumors of smooth muscle and neural origin.

Design: We examined 40 samples from 36 cases of GIST (12 benign, 13 low malignant potential [LMP], 2 high risk and 13 malignant), 10 tumors of smooth muscle origin (2 leiomyomas, 4 low grade and 4 high grade leiomyosarcomas) and 7 nerve sheath tumors (2 schwannomas, 2 neurofibromas-1 solitary and 1 diffuse, 1 low grade and two high grade malignant peripheral nerve sheath tumors [MPNST]) for the expression of ezrin using a mouse monoclonal antibody (Sigma) and the Envison detection system (DAKO). Intensity of staining was scored visually as 0 to 3+.

Results: 34 samples of GIST were strongly 3+ positive, 3 were 1+ positive, and three were negative for ezrin. The latter included one each of benign, LMP, and malignant GIST's. Benign smooth muscle, both leiomyomas and 3 out of 4 low grade leiomyosarcomas were negative while one low grade leiomyosarcoma with necrosis was 2+ positive. Four of 4 high grade leiomyosarcomas were 2+ to 3+ positive for ezrin. Normal peripheral nerve and both schwannomas were negative, 2 neurofibromas and 1 low grade MPNST were 1+ to 2+ positive and 2 high grade MPNST were 3+ positive for ezrin.

Conclusions: Ezrin appears to be constitutively expressed in Cajal cells and in most GIST's, and does not correlate with increasing aggressiveness. Potential interaction of ezrin with the c-kit pathway has not been previously investigated. Ezrin is not expressed in normal smooth muscle or peripheral nerve, and both smooth muscle and nerve sheath tumors show a correlation of ezrin expression with aggressiveness and tumor grade. Ezrin may have a role in aggressive behavior and metastasis in smooth muscle and nerve sheath tumors.

79 Histopathologic Abnormalities in Specimens from Orthopedic Spine Surgeries

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Background: Most histopathologic studies of degenerative spine disease were conducted on autopsy cases or small selected groups of surgical specimens. Tissue obtained during orthopedic surgeries (spine surgeries done for extradural, not for dural or intradural processes) is frequently received as a routine specimen in the daily practice. The frequency of different degenerative changes is, however, not well defined.

Design: 949 specimens obtained from orthopedic spine surgeries in the three years from 2000 to 2002 were reviewed retrospectively. The cases included 331 cervical, 46 thoracic, 576 lumbar and 5 of unknown location. Cases with significant findings, including degenerative changes (chondrocyte clusters, neovascularization of disc material, synovial tissue, crystal deposition) and other defined pathologic changes were further evaluated. The age distribution of the patients with and without these changes was analyzed. Chi squared analysis was performed to evaluate statistical significance. Representative cases were stained for CD44 and CD68.

Results: 14 cases (1.4%) were clinically identified synovial cysts (all lumbar). Reactive synovial tissue as a degenerative change is seen in connective tissue, fibrocartilage and yellow ligament in 4.6% of cases (1.2% cervical / 4.3% thoracic / 6.7% lumbar, p<.001). CD44 and CD68 staining confirms that the observed cells are synovium and not purely vascular proliferation. Regenerative chondrocyte clusters are found in 16.9% of cases (9.3% / 2.2% / 21.7%, p<.001), neovascularization in 4.5% (0.6% / 0.0% / 7.2%, p<.001). Calcium pyrophosphate crystals are present in 2.7% (1.5% / 0.0% / 3.7%, p>.05). 28 cases of metastatic tumor are found (0.6% / 41% / 0.8%, p<.001), all clinically suspected. Inflammatory changes including processes like osteomyelitis and foreign body reaction are present in 1.6% (15 cases), all clinically suspected. The age distribution for patients with any one of the described changes did not differ significantly from that of the patients without the respective change.

Conclusions: We assessed the frequency of different pathologic changes in a large number of spine surgery cases. Neovascularization (7.6%), synovial proliferation (4.6%), synovial cysts (1.4%) are more common in lumbar disc material. Synovial metaplasia outside the entity of synoival cyst has not previously described as a degenerative change in the spine. Calcium pyrophosphate deposition (2.7%) show no predisposition for one of the segments of the spine. Metastatic lesions to the spine are overwhelmingly more prevalent in the thoracic area.

80 FISH as an Ancillary Diagnostic Tool for the Diagnosis of Ewing Sarcoma/PNET in Routinely Processed Tissue

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Background: Ewing sarcoma (ES) and primitive neuroectodermal tumor (PNET) are diagnostically challenging small round cell tumors (RCTs) with characteristic translocations that give rise to fusion genes between *EWS* and a member of the Ets family of transcriptional regulators, most commonly *FLI1*. These translocations help distinguish ES/PNET from other RCTs in the differential diagnosis and are detectable by both reverse transcriptase polymerase chain reaction (RT-PCR) and fluorescence in-situ hybridization (FISH) in routine formalin-fixed paraffin-embedded (FFPE) specimens. However, the former can be challenging and is associated with decreased sensitivity when compared with frozen tissue. Commercial FISH probes have recently become available, but have yet to be tested in a clinical setting.

Design: In the current study, we compared FISH using two design strategies: 1) previously published homemade FISH fusion probes for EWS and FLI-1 (FISH-F) and 2) a commercial EWS break apart probe set (Vysis Inc., Downers Grove, IL) (FISH-BA). Archival blocks from 67 RCTs were retrieved for further study: 27 ES/PNETs, 10 small cell carcinomas, 8 neuroblastomas, 7 desmoplastic small round cell tumors, 7 alveolar rhabdomyosarcomas, 4 RCTs NOS, 2 infantile fibromatosis/fibrosarcomas, and 1 malignant teratoma with a PNET component.

Results: Hybridizations were interpretable in 60 (90%) cases, with signals in the remainder being absent or weak. Sensitivities and specificities for both assays were 90% and 100% and there was perfect concordance between them. However, the FISH-BA method was considerably easier to interpret than FISH-F and was less likely to yield signal counts of borderline significance.

Conclusions: FISH is a sensitive and reliable ancillary technique to provide support for the diagnosis of ES/PNET in FFPE tissue. The commercial break apart probe set is more readily available and easier to interpret. RT-PCR studies are currently in progress and will be compared with the FISH data.

81 TGF-ß1 Drives Partial Myofibroblastic Differentiation in Chondromyxoid Fibroma

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Background: Chondromyxoid fibroma is a rare benign cartilaginous bone tumor composed of lobules of chondromyxoid matrix surrounded by cellular areas, composed of spindle shaped cells. Previous ultrastructural and immunohistochemical studies have suggested the presence of myofilaments and smooth muscle actin in the neoplastic cells. Furthermore, articular chondrocytes have recently been shown, both in vivo and in vitro and under certain conditions (mainly TGF-ß1 stimulation), to express smooth muscle actin. The aim of this study was to further investigate the expression of myogenic markers in chondromyxoid fibroma and evaluate a possible role of TGF-ß1.

Design: Twenty samples (18 primary lesions and 2 recurrences) from twenty patients were studied. Immunohistochemistry was performed using antibodies against muscle specific actin (MSA), smooth-muscle actin (SMA), desmin, h-caldesmon, calponin and TGF-ß1.In porder to elucidate pathways involved in TGF-ß, driven myofibroblastic differentiation, we studied expression of fibronectin and PAI-1. Furthermore, ultrastructural studies were performed , including immuno-electron microscopy, using antibodies against MSA and SMA.

Results: Neoplastic cells were positive for MSA, SMA and negative for desmin, caldesmon and calponin. Spindle cells were diffusely positive in all samples while positive chondroblast-like cells were few in 3 cases. Ultrastructurally, neoplastic cells showed myofilaments and rare dense bodies in a spectrum, being more prominent in spindle cells and less so in chondroblast-like cells. No fibronexus was found. Immuno-electron microscopy confirmed the actin nature of these myofilaments. All the neoplastic cells were diffusely positive for TGF-ß1.

Conclusions: In conclusion, in our view, chondromyxoid fibroma shows partial myofibroblastic differentiation. This phenotype is, as shown both by immunohistochemistry and (immuno) electron microscopy, presumably driven by co-expression of TGF-ß1.

82 Array-CGH Analysis of Conventional Central Chondrosarcomas Points to Chromosome 6 and 12 Involvement in Tumor Progression

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Background: Enchondromas are the benign counterparts, and sometimes precursors, of conventional central chondrosarcomas. They can occur solitary or multiple in the context of Ollier disease (OD). Tumor progression of chondrosarcomas can be observed, whereby recurrences can be of higher grade compared to the primary tumor. Previous LOH-studies and cytogenetics did not reveal specific genetic changes, though the number of apparently non-tumor specific chromosomal aberrations increased with grade. To elucidate the genetic events, array-CGH was performed using well-defined enchondromas and central chondrosarcomas, both looking for alterations specific for chondrosarcoma, as well as related with progression. Tumors from patients with OD were included to identify putative genetic changes, since an OD specific mutation in PTHR1 was reported, although not confirmed by us.

Design: DNA was isolated from 21 frozen primary tumor samples (3 enchondromas [2 with OD], 7 grade 1, 7 grade 2 [2 with OD] and 4 grade 3 chondrosarcomas). Samples were hybridized on an array-CGH, containing >3500 PAC/BACs clone set from the Sanger Institute. ANOVA, T-test and clustering were used for analysis.

Results: No specific alterations were found for central chondrosarcoma. Although small amplifications and deletions (1-3 adjacent clones) were present in some enchondromas and grade 1 chondrosarcomas, the alterations were mainly found in the high-grade samples. In 6 samples (2 enchondromas and 4 grade 1 chondrosarcomas) no alterations were found. Hierarchical clustering, using only data from chromosome 6, separated grade 3 tumors from others. Comparing grade 2 and 3 tumors revealed several genome regions with loss or gain. A remarkable association was found between amplification of a ~ 2Mb region on chromosome 12 and progression. No specific genomic alterations were observed comparing follow up data independently of grade or comparing OD against non-OD cases.

Conclusions: The genomic alterations found in central chondrosarcomas are random, excluding the role of common genome alteration in the development of central chondrosarcomas. Gross genomic changes were mainly found in high-grade central chondrosarcomas with random losses and gains. However, alterations of chromosomes 6 and 12 were found to be associated with progression. No genomic alterations were specific for OD, thereby excluding a common amplification or deletion being causative.

83 cDNA Microarray Analysis of Conventional Central Chondrosarcomas Identifies Genes Associated with Tumor Progression, Not Related to Ollier Disease

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Background: Conventional central chondrosarcomas are malignant cartilaginous tumors, centered in the medulla of bone. A number arise secondary to enchondromas, which can be solitary or multiple in Ollier disease (OD). Sporadic and OD related central chondrosarcomas show peridiploidy and limited LOH and hence little is known about the genomic background. Since recurring chondrosarcomas may progress in grade, our goal was to elucidate signaling pathways involved in central chondrosarcoma progression. In addition, some claimed PTHR1 mutations in 2 of 6 OD patients, which we could not confirm in 31 patients. Our second goal was thus to investigate putative differences in gene expression between solitary and OD related tumors.

Design: We performed genome wide cDNA expression analysis using a 9k cDNA array enriched for cartilage specific cDNAs. RNA was isolated from enchondromas (n=3, 2 with OD), chondrosarcomas grade 1 (n=7), grade 2 (n=7, 3 with OD) and grade 3 (n=5) and normal resting cartilage (n=2). Each sample was hybridized against RNA from a common reference sample. Hierarchical clustering, t-tests, global testing and Significance Analysis of Microarrays were used for data analysis. Verification was done by quantitative realtime PCR (qPCR) or immunohistochemistry.

Results: In general, a decrease of cartilage specific extracellular matrix genes (COL2A1, COL9A2, PLOD3), an increase of glycolysis-associated genes (ALDOA) and a decrease of oxidative phosphorylation-related genes (complex I and III) was found in high-grade tumors as compared to low grade ones. Confirmation analysis was done by qPCR analysis of PLOD3 and ALDOA. OD cases revealed almost similar expression profiles as solitary tumors. JunB was one of the most differentially expressed genes (p=0.006). Immunohistochemistry confirmed the small difference found in the specific samples from the array, although on a larger series no difference in expression was present between OD (n=13) and solitary tumors (n=76).

Conclusions: Upon chondrosarcoma progression, matrix-associated genes are down regulated, reflecting the histology of high-grade tumors. Moreover, glycolysis associated genes are upregulated and oxidative phosphorylation is decreased, suggesting an adaptation in energy supply upon progression towards higher grade. In addition, OD and solitary cases showed similar gene expression profiles, suggesting that the same signaling pathways are affected in their histogenesis.

84 Nestin Expression in Malignant Peripheral Nerve Sheath Tumor (MPNST) and Other Soft Tissue Tumors

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Background: MPNST is a malignant tumor originated from Schwann cells. Although it is sometimes difficult to diagnose MPNST by H&E, there is no useful diagnostic marker for it. S-100 protein is regarded as one of the marker of MPNST, however it is positive in less than 50% of MPNSTs. Nestin is one of the intermediate filaments expressed in neuroectodermal stem cells and skeletal muscle progenitor cells. Nestin is also detected in peripheral nervous tumors. The aim of the current study is to evaluate the diagnostic utility of Nestin in MPNST

Design: Formalin-fixed, paraffin-embedded sections were prepared from 34 samples (31 cases) of MPNSTs and 78 other soft tissue tumors, including 10 neurofibromas, 10 schwannomas, 10 leiomyomas, 19 leiomyosarcomas, 5 rhabdomyosarcomas, 10 liposarcomas, 7 carcinosarcomas and 7 malignant fibrous histiocytomas. Sections were stained with H&E and with the antibodis (Nestin, S-100,PGP.9.5 and CD56) using a biotin free detection system. The cases showing more than 50% positivity for each antibody were regarded as positive.

Results: Nestin showed granular cytoplasmic or subplasmalemmal linear staining pattern. The immunohistological results are summarized in the following table.

	Nestin	S100	PGP9.5	CD56
MPNST	91%(31/34)	29%(10/34)	71%(24/34)	18%(6/34)
Schwannoma	60%(6/10)	100%(10/10)	90%(9/10)	50%(5/10)
Neurofibroma	10%(1/10)	80%(8/10)	10%(1/10)	0%(0/10)
Leiomyoma	0%(0/10)	0%(0/10)	0%(0/10)	4%(4/10)
Leiomyosarcoma	74%(14/19)	0%(0/19)	21%(4/19)	63%(12/19)
Rhabdomyosarcoma	80%(4/5)	0%(0/5)	40%(2/5)	60%(3/5)
Liposarcoma	20%(2/10)	0%(0/10)	10%(1/10)	10%(1/10)
Carcinosarcoma	57%(4/7)	0%(0/7)	43%(3/7)	57%(4/7)
MFH	43%(3/7)	0%(0/7)	57%(4/7)	0%(0/7)

Conclusions: Our present study showed that Nestin is a sensitive marker for MPNST, although it is not highly specific. Nestin could be a useful diagnostic marker for MPNST in combination with other markers. Interestingly, some of malignant tumors were positive for Nestin, while benign tumors except schwannomas were negative. Nestin could be a adjunct marker for malignant soft tissue tumors.

85 Angiomatoid Fibrous Histiocytoma of Bone

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Background: Angiomatoid fibrous histiocytoma (AFH) is a rare soft tissue tumor, primarily affecting children and young adults. It is most frequently encountered in the subcutaneous soft tissue of the trunk and lower extremities. We present the clinicopathologic features of three patients with a primary AFH of bone.

Design: Clinicopathologic and radiographic data of three patients with AFH of bone available in our consultation files were reviewed.

Results: The tumors affected a 7 year-old girl, 8 year-old boy, 47 year-old male and involved the right mandible, right proximal humerus and right proximal ulnar shaft respectively. One of the patients had associated right axillary and supraclavicular lymphadenopathy, fever, episodes of nausea and vomiting, and 6-pound weight loss in 6 weeks. Radiographically all tumors were destructive intramedullary lesions with complete cortical penetration and extension to the paraosseous soft tissue. There was no evidence of matrix production. Histologically, the tumors were composed of solid areas of mesenchymal cells with histiocytoid appearance alternating with multiple blood-filled spaces, and lymphoid aggregates at the periphery. All tumors showed microscopic evidence of complete cortical penetration and extension to the paraosseous soft tissue. Inmunohistochemically, the tumor cells were positive for vimentin and negative for histiocytic and endothelial markers. The humeral lesion was initially treated by curettage and the patient developed recurrences 3 and 9 months after the original procedure. Finally, it was treated with the proximal humeral resection. The ulnar lesion represented the recurrence 7 years after the original curettage. No follow-up data was available for the case involving the mandible.

Conclusions: AFH is a distinct mesenchymal neoplasm, which rarely affects bone and exhibits aggressive destructive growth with high potential for local recurrence.

86 Angiostatin Receptor Annexin II in Vascular Tumors

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Background: Inhibitors of angiogenesis such as angiostatin are increasingly being used for therapy of human tumors, targeting the tumor neovasculature, and have had mixed success. Annexins are a multigene family of calcium and phospholipid membrane binding proteins implicated in membrane transport events. Annexin II (ANX2) is a 36 kDa cell surface receptor for angiostatin. We hypothesized that, like normal vascular endothelium, vascular neoplasms would express ANX2, implying the potential usefulness of angiostatins in the therapy of this family of soft tissue tumors.

Design: Various vascular tumors were selected and the diagnoses were reviewed. 38 total cases tested included: hemangiomas - capillary [4], cavernous [6], lobular capillary [LCH, 6]); intramuscular hemangioma [3], spindle cell [1], and epithelioid hemangioma [4]; epithelioid hemangioendothelioma [3], and angiosarcoma [7], 4 of which were epithelioid. Four [4] angiolipomas were also selected. Annexin II antibody (Zymed) was used (1/50 dilution, Ventana ES autostainer). Reactivity location (cytoplasmic, nuclear, membrane), intensity (1+/2+/3+), and quantity (focal, diffuse) was recorded.

Results: In normal tissue, ANX2 reactivity was noted in dermal fibroblasts, endothelium of blood and lymphatic vesels, myofibroblasts, and pericytes, along with epithelial structures. ANX2 was identified in all tumors except one capillary hemangioma (37/38+, 97%). The reactivity was diffuse in all cases except 2 (focal). Staining was strong 2+ or 3+ in 87% of cases; 5/38 had focal reactivity, all benign tumors. All lesions exhibited cytoplasmic reactivity, and membrane staining was appreciated in most; no nuclear staining was seen. In lesions with endothelium and pericytes (LCH), both cell types were positive. Membrane positivity was easily identifiable in the 4 epithelioid angiosarcomas.

Conclusions: To our knowledge, this is the first demonstration of an angiostatin receptor (ANX2) in vascular endothelial tumors including agiosarcoma. The presence of diffuse and strong reactivity signified the absence of any down-regulation of this receptor in both benign and malignant tumors. ANX2 reactivity may be the basis for treatment of a variety of benign tumors especially in pediatric patients. Further, the presence of ANX2 may offer a new and potentially less toxic therapy for angiosarcoma, which is frequently aggressive and often fatal.

87 Detection and Genotypic Identification of Characteristic Fusion Gene Transcripts from Formalin-Fixed Soft Tissue Tumor Specimens Using Multiplex RT-PCR/Capillary Electrophoresis

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Background: Minimally invasive biopsy strategies, which limit the amount of tissue submitted for pathologic review, have made molecular diagnostic techniques an increasingly important aspect of soft tissue tumor diagnosis. The detection of characteristic translocations, or their chimeric transcripts, can aid in the accurate diagnosis of these tumors, especially when architectural features are suboptimally represented. However, the lack of available fresh tissue for nucleic acid extraction makes the detection of such markers challenging in many cases.

Design: We have developed a set of assays combining one-step multiplex RT-PCR with capillary electrophoresis to detect transcripts from the characteristic translocations in Ewing's sarcomas (*EWS/FLI1, EWS/ERG*), alveolar rhabdomyosarcomas *(PAX3/FKHR, PAX7/FKHR)* and desmoplastic small round cell tumors *(EWS/WT1)*. Small amplicons from tumor specific chimeric transcripts as well as control gene transcripts are differentially labeled with fluorophores, allowing detection and genotypic classification by capillary electrophoresis. We analyzed RNA extracted from 34 archival formalin fixed, paraffin embedded tissue specimens retrieved from our Department of Pathology files.

Results: The assays detected and correctly genotyped chimeric transcripts from 10/13 (77%) Ewing's sarcomas (9 *EWS/FLI1*, 1 *EWS/ERG*), 8/9 (89%) alveolar rhabdomyosarcomas (all *PAX3/FKHR*), and 2/2 desmoplastic small round cell tumors (both *EWS/WT1*). In the case of the transcript negative alveolar rhabdomyosarcoma, cytogenetic analysis also failed to demonstrate a translocation. DNA sequencing of representative amplicons confirmed the results obtained by capillary electrophoresis. None of the assays identified chimeric transcripts in 9/9 malignant peripheral nerve sheath tumors or 1/1 epithelioid sarcoma. Additionally, each tumor type was cross-analyzed by each of the different assays, and in no case was an unexpected transcript type identified, confirming the specificity of the assays.

Conclusions: The combination of one-step multiplex RT-PCR with capillary electrophoresis provides a rapid and accurate method for the detection of tumor-specific chimeric transcripts. Their suitability for use with fixed tissue specimens makes these assays a valuable adjunct to the histologic diagnosis of soft tissue tumors.

88 Detection and Genotypic Classification of *SYT/SSX* Transcripts from Synovial Sarcomas Using Multiplex RT-PCR/Capillary Electrophoresis

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Background: Synovial sarcomas are rare, highly malignant tumors of soft tissue. There are two histological subtypes: monophasic, composed entirely of spindle cells, and biphasic, containing both spindle cells and epithelial cells arranged in gland-like structures. Diagnosis of these tumors based on histology can be challenging, particularly when minimal biopsy specimens are presented to the pathologist. Demonstration by molecular methods of *SYT/SSX* transcripts, resulting from the t(X;18) which characterizes these tumors, is a useful adjunct for diagnosis in these situations. Our goal was to establish a rapid, sensitive and specific assay for the detection of *SYT/SSX* transcripts, from fresh or fixed tissue specimens, that can be routinely performed in a clinical laboratory.

Design: We have developed an assay which combines one-step multiplex RT-PCR with capillary electrophoresis to detect and genotype the *SYT/SSX* transcripts characteristic of synovial sarcomas. Small amplicons from tumor-specific chimeric transcripts as well as control gene transcripts are differentially labeled with fluorophores in a single PCR tube, allowing detection and genotypic identification by capillary electrophoresis. We analyzed RNA extracted from 32 previously diagnosed archival formalin fixed, paraffin embedded tissue specimens retrieved from our Department of Pathology files.

Results: The assay detected chimeric transcripts from 17/22 (77%) synovial sarcomas; all 5 assay negative specimens yielded no intact RNA as evidenced by lack of amplification of an equally sized fragment from the *GAPD* transcript. *SYT/SSX1* transcripts were identified in 12/17 cases (9 biphasic, 3 monophasic), while 5/17 cases were positive for an *SYT/SSX2* transcript (1 biphasic, 4 monophasic). Only one transcript type was identified in each case. Representative amplicons were sequenced and confirmed the genotyping results obtained by capillary electrophoresis. Chimeric transcripts were not detected in 9/9 malignant peripheral nerve sheath tumors or 1/1 epithelioid sarcoma.
Conclusions: One-step RT-PCR combined with capillary electrophoresis is a rapid and accurate method for the detection and genotyping of *SYT/SSX* transcripts from fixed tissue specimens. We discuss the advantages of an assay combining RT-PCR with capillary electrophoresis as an adjunct to the histologic diagnosis of synovial sarcomas.

89 CSF1R Expression in Soft Tissue Tumors

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Background: Receptor tyrosine kinases are cell surface growth factor receptors that transduce a wide variety of signals including those for growth, differentiation, and survival, and are known to play an early role in oncogenesis. A number of tumor types harbor activating mutations in receptor tyrosine kinases. The mutation status in these receptors correlates with response to small molecule tyrosine kinase inhibitors in some tumors. These include gastrointestinal stromal tumor (GIST), dermatofibromasarcoma protuberans (DFSP), and a subset of lung adenocarcinomas. We have recently surveyed the expression of three receptor tyrosine kinase type III family members in soft tissue tumors. We now report the expression of a fourth member of the tyrosine kinase type III family, *CSF1R* , in these tumors.
Design: We examined over 500 soft tissue tumor cases covering over 50 soft tissue tumors using in situ hybridization and tissue microarrays. We compared *CSF1R* expression to *KIT* , *PDGFRA* , and *PDGFRB* expression.
Results: *CSF1R* was expressed in a small subset (10-30%) of numerous tumor types, including leiomyosarcomas, malignant fibrous histiocytomas, and osteosarcomas. Interestingly, all 9 tenosynovial giant cell tumors expressed *CSF1R* , in both the mononuclear and the multinucleated giant cells. Finally, *CSF1R* was frequently co-expressed with *PDGFRB* .
Conclusions: *CSF1R* demonstrates tumor specific expression and is highly expressed in tenosynovial giant cell tumors. The distribution of receptor tyrosine kinase co-expression may provide insight into the potential interaction between receptors.

90 The Utility of β-Catenin in the Distinction between Fibromatosis and Its Morphologic Mimics

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Background: The adhesion molecule β-catenin, is a member of the catenin family normally found in a sub-plasmalemmal distribution in human cells. This localization appears to depend on an intact adenomatous polyposis coli (APC) pathway, alteration of which causes phosphorylation and degradation of β-catenin leading to its abnormal nuclear localization . APC alteration occurs in a number of conditions, including fibromatosis. Investigators were able to immunohitochemically demonstrate the utility of β-catenin in discriminating between mesenteric fibromatosis and morphologically similar entities (Am J Surg Pathol. 2002;26(10):1296-301). Nonetheless, to our knowledge, the expression profile of β-catenin has not been expanded to include a wider spectrum of morphologically similar reactive lesions.
Design: This study aimed at: (a) determining the spectrum of β-catenin expression in the most common reactive spindle cell lesions with desmoid-like morphology; and (b) validating the potential utility of β-catenin as a marker distinguishing fibromatosis from reactive mimicks. Paraffin-embedded lesions were tested via immunohistochemistry with β-catenin antibody (clone 14, Transduction Laboratories/ BD, Lexington, KY). Deparaffinized sections were subjected to epitope retrieval via steaming in citrate (pH = 6) buffer for 40 minutes, followed by 20-minute cooling down. Polymer-based detection system (Envision, DakoCytomation, Carpenteria, CA) was employed. Only nuclear expression was considered positive.Fifty six cases include abdominal fibromatoses (n = 19), dermal scars (n = 11) ventral hernia sacs (n = 13) and reactive fibrosis secondary to miscellaneous conditions (n = 13).
Results: Nuclear expression of β-catenin is noted on all fbromatoses (100%). Except for rare positive nuclei, none of the ventral hernias or secondary fibrosing lesions were positive. Of note, One scar lesion (9%) shows strong nuclear signal. Lesions without nuclear signal showed either membranous expression pattern (non-mutated protein) or total absence of signal.
Conclusions: These results indicate that (a) nuclear expression of β-catenin in fibromatosis-like reactive conditions is extremely rare, and (b) β-catenin is extremely useful for the distinction between fibromatosis and morphologic mimics, with somewhat limited utility in scar lesions. Additional studies on the latter using molecular tools may provide insightful information on the pathogenesis of various scarring conditions.

91 Expression of Tumor Suppressor Gene Ini1 Is Down-Regudated in Epithelioid Sarcoma (ES), Biologic and Diagnostic Implications

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Background: *Ini1/hsnf5* gene at 22q11.2 encodes Ini1 protein, a chromatin remodeling factor associated with the SWI/SNF complex which modifies chromatin condensation to coactivate various transcriptional factors in yeast. In humans, this gene has been found to be mutated in malignant rhabdoid tumor (MRT) and some other tumors and has been implicated as a tumor suppressor gene. The *Ini1/hsnf5* gene mutation leads to the down regulation of protein expression in these tumors. ES and MRT are two very distinct soft tissue entities, but the epithelioid and rhabdoid morphology characteristic of these two tumors respectively, could sometimes mimic each other. Ini1 expression in ES has not been evaluated.
Design: Paraffin sections of 22 ES were stained with mAb Ini1/BAF47 (clone 25, 1:250, BD Transduction Labs) after 8 min microwave antigen retrieval in 1X TRS (PH 9.5). Vector ABC kit (Vector Labs) was used for detection in a DAKO autostainer. In addition, Ini1 was also evaluated in 21 spindle cell sarcomas [2 clear cell sarcomas, 4 leiomyosarcomas, 3 monophasic synovial sarcomas (MSS), 3 dermatofibrosarcoma protuberans (DFSP) and 9 others]; 8 round cell sarcomas (2 EWS, 2 neuroblastomas, 4 desmoplastic round cell tumors); 8 carcinomas (1 colon, 1 renal, 1 prostate, 2 thryoid, 3 breast); 5 melanomas; and normal tissue of prostate, tonsil, kidney, breast, cerebellum and colon.
Results: High level Ini1 expression was widely detected in all normal tissues, carcinomas melanomas, round cell sarcomas and all but three spindle sarcomas tested (negative in 1 MSS and decreased in 1 MSS and 1 DFSP). In contrast, 16 of 22 (73%) ES were negative for Ini1. Ini1 reactivity was weak and focal in 5 of the remaining 6 ES.
Conclusions: Ini1 expression is significantly down-regulated in ES on immunohistochemical level. The loss of Ini1 expression in ES might be due to gene mutation as seen in MRT and suggests an oncogenic role of SWI/SNF chromatin remodeling complex in the development of ES. ES should be added to the short list of tumors with abnormal Ini1 expression. In addition to MRT, Ini1 immunostaining might be useful in differentiating ES from its mimics, although this marker is not useful in separating MRT and ES.

92 D2-40 Is a Useful Marker for Subtyping Rhabdomyosarcoma (RMS)

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Background: D2-40 is a monoclonal antibody against the M2A antigen, a MW 40K O-link sialoglycoprotein originally identified in fetal testes. Recently D2-40 immunoreactivity has been detected in lymphatic endothelium and mesothelium, and has been used as a marker for germ cell tumor, mesothelioma and lymphatic vascular neoplasm. Variable D2-40 reactivity has also be reported in spindle cell sarcomas. We have recently observed cytoplasmic D2-40 reactivity in fetal skeletal muslce and stressed adult skeletal muscle. D2-40 reactivity has not been evaluated in RMS and other round cell sarcomas
Design: 23 RMS (9 alveolar, 13 embryonal), 11 EWS/PNET, 13 neuroblastomas (NB), 8 desmoplastic small round cell tumors (DSRCT) were evaluated for D2-40 reactivity. In addition to morphology, the diagnoses were confirmed by immunostaining for myogenic, epithelial and neuroectodermal markers and RT-PCT analysis for *PAX 3 or 7-FKHR, EWS/Fli-1, and/or EWS/WT-1* gene products at the time of diagnosis. Paraffin sections were boilded in EDTA before incubation of D2-40 (1:200, Signet). EnVision+ (DAKO) was used for immuodetection in a DAKO autostainer. D2-40 immunoreactivity was evaluated semiquantitatively.
Results: D2-40 immunoreactivity was detected in none of the 9 alveolar RMS, 9 of 13 (69%) embryonal RMS, 3 of 11 (27%) EWS/PNET and none of 8 DSRCT. No D2-40 reactivity was detected in any neoplastic small neuroblasts in NB. Focal D2-40 reactivity was seen in mature ganglion cells and schwannion stroma present in 3/ 13 cases. Of the 9 cases of D2-40-positive embryonal RMS, the cytoplasmic immunostaining was strong in >50% of tumor cells in 5, moderate in >20% in 3 and weak and focal in 1 case.
Conclusions: Morphologic evaluation alone is difficult to classify RMS into alveolar and embryonal subtypes, which has significant clinical implication. The current available myogenic markers are useful to differentiate RMS from other round cell tumors but have no use in differentiating alveolar vs. embryonal RMS. Currently, RMS could only be subtyped reliably by detecting *PAX3 or PAX7-FKHR* fusion gene products in alveolar RMS by Rt-PCR. Because of the striking difference in D2-40 reactivity between alveolar (100% negative) and embryonal RMS (74% positive), for the first time, pathologists might be able to use immunohistochemical method to subtype RMS.

93 The Alveolar Soft Part Sarcoma Marker TFE3 Is Also Frequently Expressed in Granular Cell Tumor

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Background: Aberrant nuclear immunoreactivity for TFE3 is a sensitive marker of tumors with known translocations involving the *TFE3* gene at Xp11.2 (Am J Surg Pathol 2003; 27: 750-761). One of these tumors is alveolar soft part sarcoma (ASPS) that expresses the ASPL-TFE3 fusion protein due to a der(17)t(X;17)(p11.2;q25). However, TFE3 immunoreactivity has been reported in rare tumors without known *TFE3* gene fusions. One such tumor that also represents one of the main histological differential diagnoses of ASPS is granular cell tumor (GCT). In a prior study, we observed moderate to strong nuclear labeling for TFE3 in some GCTs. We have now

performed a more thorough evaluation of the prevalence and degree of TFE3 immunoreactivity in GCT .

Design: A tissue microarray (TMA) was assembled from formalin-fixed and paraffin-embedded tissue blocks of 37 cases of GCT and one case of ASPS, and contained four 0.6 mm cores of tumor tissue from each case. Of the patients with GCT, 19 were male, and 18 were female with an average age of 46.3 yr (range 9 – 71 yr). The primary sites were skin (20), tongue (4), soft tissue (6), vulva (2), breast (2), esophagus (2), and palate (1). TFE3 immunostains were performed and scored as described in the above study.

Results: Nuclear immunoreactivity for TFE3 was observed in 34 of 37 (92%) GCT, and in the positive control ASPS case. Of the 34 TFE3 positive GCTs, 11 (32%) showed strong (3+) and 23 (68%) showed moderate (2+) nuclear labeling. The majority of TFE3 positive cases (30/34, 88%) showed diffuse reactivity with 75 – 100% nuclei positive while the remainder show reactivity in 30 – 75% of nuclei. TFE3 nuclear labeling was not seen in control normal tissues (skin, liver, adipose tissue, skeletal muscle, breast) or other neoplasms (schwannoma, fibroadenoma, phylloides tumor, rhabdomyosarcoma, melanoma) included in the TMA.

Conclusions: The prominent and frequent nuclear TFE3 immunoreactivity of GCTs precludes its use in discriminating GCTs from ASPS and is so far unique among tumors without known TFE3 translocations. Its basis may warrant further investigation.

Breast

94 ZO-1 and Occludin - Novel Markers of Lobular Carcinoma of Breast

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Background: Expression of E-cadherin, an adhesion molecule, has been used to distinguish lobular carcinoma from ductal carcinoma of breast. During the development of cell-cell junctions cadherin associate with a number of proteins including tight junction proteins Zonula Occludens- 1 (ZO-1) and Occludin. These proteins normally localize to the apical aspect of the breast epithelial cells. Expression of ZO-1 is associated with gland formation in breast cancer and has been reported in low grade invasive ductal carcinomas. As lobular carcinoma does not exhibit gland formation, we hypothesized that the distribution of these polarity related proteins might be altered in lobular carcinoma and could be used as markers of this disease. In this study we examine the expression of ZO-1 and Occludin in lobular cancer of breast and differentiating it from ductal carcinoma of breast.

Design: Archival tissue specimens from 20 patients having lobular carcinoma of which 12 had a lobular carcinoma in-situ breast cancers were analyzed for the expression of Occludin (Zymed, pre-diluted) and ZO-1 (Zymed, pre-diluted) by immunohistochemistry.

Results: Both ZO-1 and Occludin showed an apical zonal expression in normal luminal epithelial cells and this was seen in all cases and served as an internal control. ZO-1 expression was also noted in endothelial cells.

ZO-1 and Occludin expression was completely lost in invasive component in 16/20 cases (80%) of invasive lobular carcinoma. Loss of normal apical distribution was seen in the other four. This was in the form of diffuse cytoplasmic expression in 3 cases and focally membranous expression in one case. ZO-1 and Occludin expression was completely lost in 11/12 (92%) cases of lobular carcinoma in-situ and showed altered distribution in the form of diffuse cytoplasmic staining in one case.

Conclusions: Loss of cellular polarity as indicated by expression of tight junction proteins ZO-1 and Occludin is an early event in lobular carcinoma. It is seen in both invasive and in-situ lesions. Loss and/or abnormal localization of adhesion proteins could give rise to the diffuse pattern of growth characteristic of lobular cancer of breast. Since alteration in normal distribution was seen in all the lesions examined, it appears that ZO-1 and Occludin could be good markers for the diagnosis of lobular carcinoma. Further studies are ongoing to assess the utility of these markers in distingiushing lobular carcinomas from ductal lesions.

95 Lymphangiogenesis Does Not Occur in Breast Cancer

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Background: Lymph node metastasis is one of the best indicators of prognosis in breast cancer. Although the importance of angiogenesis in hematogenous spread of breast cancer is well established, little is known about the invasion and generation of lymphatic vessels. This is due to lack of markers that specifically identify lymphatic vessels. VEGF-C and LYVE-1 have been previously used for this purpose but they lack specificity and often do not distinguish lymphatic endothelium from blood endothelial cells. Recently, D2-40 has been described as a novel specific marker for identification of lymphatic vessels. In this study we used dual immunohistochemistry (with D2-40 and PCNA) to identify and study lymphangiogenesis in breast cancer.

Design: Double immunohistochemistry was performed on paraffin sections from 25 patients having breast cancer for D2-40 (Signet Lab, dil 1: 40) antibody and polyclonal PCNA (dilution 1: 400). A lymphatic vessel density count was performed by noting the expression of D2-40 in the lymphatics. The lymphatic endothelial proliferation was studied by studying the expression of PCNA in the tumour cells as an internal control.

Results: Lymphatic vessels as identified by D2-40 expression were seen in the peritumoral area. However, lymphatics were not identifed within invasive tumors, except in areas adjacent to pre-existing ducts and lobules. The lymphatic endothelial cells did not show any expression of PCNA indicating minimal or no proliferative activity. This was in contrast to strong expression in adjacent tumor cells, which served as internal control.

Conclusions: Whether breast tumor cells co-opt and invade existing lymph vessels or induce proliferation of new lymphatic vessels is not known.Our findings suggest that lymphangiogenesis does not occur in breast cancer and it is likely that breast cancer cells utilise pre-existing lymphatics for metastasis. In contrast to angiogenesis, lymphangiogenesis does not seem to play a major role in the metastatic invasion of breast tumors.

96 Core Biopsy Specimens with and without Calcifications: Should They Be Submitted Separately?

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Background: Mammotome core biopsy of mammary microcalcification (MC) ia a commonly used diagnostic method. Radiologists perform core specimen radiographs to document the presence of MC. For the surgical pathologist identification of MC is critical to ensure appropriate sampling of the radiographic finding. Cores "with calcification" and cores "without calcification" are submitted in individual containers and the final pathology report contains separate diagnoses for the cores with and without MC. We reviewed pathology reports from cases of MC to investigate whether this separation was necessary.

Design: Computerized search yielded 119 11-gauge mammotome core biopsies from 110 female patients (age range 33 to 81 yrs) with MC. Cores were radiographed and separated according to the presence or absence of MC and placed in formalin containers marked "with calcification" and "without calcification". Cores were paraffin-embedded, sectioned with three levels and stained with hematoxylin and eosin. The diagnoses from the individual containers were compared and differences were analyzed.

Results: In 93 cases (78%) there was no difference in diagnosis between cores marked as "with calcification" and "cores without calcification". The majority of these cases (66%) yielded a benign diagnosis. The remaining 33% were DCIS (17), invasive carcinoma (13) or atypical ductal hyperplasia (1). In 26 cases (22%), the diagnoses were different. In 21 cores with MC and in 5 cores without MC, differences between the two diagnoses would have led to changes in patient management ($p \leq 0.001$, chi-square test). Results are summarized in Table 1.

Conclusions: In the majority of mammotome biopsies for MC (78%), separation of cores by specimen radiography did not contribute to a difference in pathologic diagnosis, regardless of the presence or absence of MC. A difference in diagnosis that would lead to a change in patient management was noted in 22%. In 10 cases, separation of cores allowed a definitive diagnosis of malignancy in cores containing MC in comparison to those without MC.

Comparison of diagnoses of mammotome core biopsy specimens with and without microcalcification

		Cores without calcification			
Cores with calcification	Non diagnostic	Benign	Atypical Hyperplasia	DCIS	Invasive carcinoma
Non diagnostic					
Benign	3	62	3*		
Atypical Hyperplasia		4	1*		
DCIS		9	3**	17	2
Invasive Carcinoma		1		1	13

*Atypical ductal hyperplasia in all cases, **2 cases atypical ductal hyperplasia, 1 case atypical lobular hyperplasia

97 Prognostic Significance of Combined Use of Histologic Grade and Ki-67 in Breast Cancer

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Background: Grading systems based in Patey-Scarff and Bloom-Richardson, later modified by Elston has been applied and validated repetitively in large series of breast cancer cases. The proliferative capacity of neoplasms is one of the most crucial variables for tumor grading. More recently, immunohistochemical quantification of Ki67 has been applied in routine study of proliferative activity in breast cancer. However, the significance of combined use of histologic grade (HG) and Ki67 is not well established.

Design: Formalin-fixed, paraffin-embedded tissues from 244 invasive breast cancers were stained with Ki67 (monoclonal MIB-1, 1:50; Dakocytomation). Cases with ≥20% of stained tumor cells were considerate as high-Ki67. In all cases, Nottingham combined histologic grade (HG) was applied. A combination based on HG and Ki67 was applied as following: low grade (HG I and low-Ki67), intermediate grade (HG I-II with high-Ki67 or HG III with low-Ki67), and high grade (HG II-III with high-Ki67). Median follow-up was 59 months (range 4-102). The univariate relationship between variables and overall survival (OS) was analyzed by the Kaplan-Meier method, and the differences were assessed by the log-rank test. All statistical manipulations were performed using the SPSS for Windows system.

Results: Tumors with HG III contained high level of Ki67 ($p<0.000$). OS rate of cases with low-Ki67 was 89% and with high-Ki67 75% ($p=0.0021$). OS rate in HG I was 92 %, in HG II 84%, and in HG III 77% ($p=0.0192$). OS rate in low-grade tumors was 94%, in intermediate-grade 85%, and in high-grade 74% ($p=0.0024$). OS rate in lymph node negative patients (n=170) was as follows: low-Ki67 97% vs. high-Ki67 82% ($p=0.0005$); HG I 97 %, HG II 93% and HG III 84% ($p=0.0237$); low-grade 100%, intermediate-grade 92% and high-grade 80% ($p=0.0012$).

Conclusions: Histological grade and immunohistochemical detection of Ki67 represent two parameters that provide prognostic information, independently of the stage of the disease. Combination of both allows a better selection of risk groups in patients with breast carcinoma and defines a group with excellent prognosis.