were selected from the autopsy files. The heart weights were collected; five micron, paraffin-embedded sections of the left ventricle were stained with Sirius Red F3BA for collagen. Multiple areas from each slide were digitized and interstitial collagen quantified using Image Pro Plus software. Collagen volume fraction was calculated and compared to collagen volume fraction from five control patients (BMI ≤ 25 kg/m^2).

Results: The ages of the patients ranged from 19 to 76 (mean 46) for the morbidly obese group and 34 to 69 (mean 56) for the control group. The BMI of the obese patients ranged from 40 to 80 kg/m^2 (mean of 51), and the control group ranged from 23 to 25 kg/m^2 (mean 24). History of hypertension was present in five, absent in four and not known in three obese patients. One control patient had hypertension. Heart weight was not significantly higher in the morbidly obese group compared to controls (370 ± 38 versus 354 ± 31, p = 0.79, respectively). Collagen volume fraction was significantly increased in the morbidly obese patients (1.6% ± 0.22) compared to lean controls (0.6% ±0.04), p < 0.05).

Conclusions: Morbid obesity stimulates cardiac interstitial collagen production. These results suggest that collagen production may contribute to the high risk of cardiac failure, especially diastolic failure, found in morbid obesity.

19 Discrepant Major Diagnosis and Unexpected Additional Diagnoses Found at Autopsy: A Comparison of University, Community, and Private Autopsy Practices

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Background: Recent studies continue to demonstrate a high disparity rate between clinical and autopsy diagnoses. Few have compared different institutional settings.

Design: We retrospectively reviewed consecutive adult autopsy results from three settings from 2002-2005: University hospital (n=86, patient age 59 ± 17 years, 40% women, 41% Black); community hospital (n=103, patient age 61 ± 15 years, 53% women, 39% Black); and private commissioned autopsies (n=55, patient age 68 ± 15 years 53% women, 33% Black). The community and private autopsies were all performed by the same prosector. Major discrepancies (autopsy findings refuting accuracy of cause of death per death certificate) and major additional findings (potentially affecting patient care but not cause of death) were determined prospectively.

Results: The rate of major discrepancies was 8% (University), 19% (community) and 31% (private). The rate of additional major findings was 27% (University), 36% (community) and 51% (private). The largest group of major discrepancies in the combined private and community setting was undiagnosed pulmonary embolus attributed to coronary disease (40%), followed by undiagnosed infection (14%), ruptured aneurysm (14%) and undiagnosed neoplasm (11%). The largest group of major discrepancies in the University setting was undiagnosed coronary artery disease (43%). Undiagnosed infections included peritonitis (2), meningitis (1), miliary tuberculosis (1), and endocarditis (1). Undiagnosed malignancies included disseminated lymphoma (1), small cell carcinoma of lung (1), urothelial carcinoma (1); meningeal melanoma (1), and biliary carcinoma (1). By univariate analysis, discrepant findings were associated inversely with length of hospital stay (p<.01) and private (p<001) and community (p<.01) settings. By multivariate anlasis, only private setting (p=.02) correlated (inversely) with rate of discrepant results.

Conclusions: We conclude that unexpected findings are not uncommon in autopsy preformed currently, especially with short hospital stay and requested privately by family. The single largest misdiagnosis remains pulmonary embolus attributed to coronary artery disease.

Bone & Soft Tissue

20 Genomic Gains of *COL1A1-PDGFB* Copies Occur in Fibrosarcomatous Transformation of Dermatofibrosarcoma Protuberans

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Background: Dermatofibrosarcoma protuberans (DFSP) is a superficial low grade sarcoma that rarely evolves into a higher grade fibrosarcoma. DFSP is genetically characterized by the unbalanced chromosomal t(17;22)(q21;q13), usually in the form of a supernumerary ring chromosome. The product of this chromosomal translocation is the chimeric gene *COL1A1-PDGFB*, which is amplified at low levels in the supernumerary ring chromosome. The aims of this study were to evaluate (1) whether additional amplification of this fusion gene occurs during the clonal evolution of DFSP into fibrosarcomatous DFSP and (2) whether there is a difference between the number of genomic copies of *COL1A1-PDGFB* between classic DFSP and DFSP areas associated with fibrosarcomatous DFSP.

Design: Ten cases of fibrosarcomatous DFSP with both DFSP and fibrosarcomatous areas and 10 cases of classic DFSP were studied. Genomic copies of *COL1A1-PDGFB* were evaluated by fluorescence in situ hybridization (FISH) using a custom designed probe for the *PDGFB* locus on 4µm thick paraffin-embedded tissue sections. Approximately 500-1000 cells were independently scored in both DFSP and fibrosarcomatous areas in each tumor.

Results: Additional gains of *COL1A1-PDGFB* locus signals were observed in 6 (of 10) fibrosarcomatous DFSP in the fibrosarcomatous areas (2-7 gene copies, median 4.2) when compared to the classic DFSP areas (2-3 gene copies, median 2.2). Four fibrosarcomatous DFSP did not show additional genomic gains of *COL1A1-PDGFB* between the two areas. No difference in the copy number of *COL1A1-PDGFB* fusion gene was observed between classic DFSP and DFSP areas of fibrosarcomatous DFSP (2-3 gene copies in each).

Conclusions: Additional genomic gains of *COL1A1-PDGFB* fusion gene is an oncogenic mechanism that can occur in the clonal evolution of a subset of DFSP into fibrosarcomatous DFSP. Overall *COL1A1-PDGFB* fusion gene copy number in the classic DFSP areas does not seem to be a major predisposing mechanism for fibrosarcomatous transformation.

21 *EWS-CREB1*: A Recurrent Variant Fusion in Clear Cell Sarcoma Associated with Gastrointestinal Location and Absence of Melanocytic Differentiation

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Background: Clear cell sarcoma (CCS) usually arises in the lower extremities of young adults and is typically associated with a t(12;22) that results in the fusion of *EWS* with *ATF1*, a gene encoding a member of the CREB family of transcription factors. CCS arising in the gastrointestinal (GI) tract is extremely rare and its pathologic and molecular features are not well defined.

Design: In the course of testing a CCS from the colon of an 81 year old woman for the *EWS-ATF1* fusion by reverse transcriptase PCR (RT-PCR), we detected a novel variant fusion of *EWS* to *CREB1*, a gene at 2q32 encoding another CREB family member highly related to ATF1. We then studied additional cases with similar features for *EWS-CREB1*.

Results: We subsequently identified two additional GI CCS with the *EWS-CREB1* fusion by RT-PCR, both arising in the small bowel, both in 42 year old women. In 2/2 cases tested, *EWS* gene rearrangement was also confirmed by FISH and the *EWS-CREB1* genomic junction fragments were isolated by long range DNA PCR. Morphologically, all 3 lesions had a solid, nested, or pseudopapillary growth pattern, with an epithelioid and uniform cytology. No melanin pigmentation was noted. By immunohistochemistry (IHC), there was strong and diffuse S100 protein reactivity, while all melanocytic markers and CD117 were negative. Neural markers, such as NSE, CD56 and synaptophysin were positive. Ultrastructurally, electrondense granules were identified in 2/2 cases but they lacked the diagnostic features of melanosomes. Finally, RT-PCR for the melanocyte-specific transcript of *MITF* showed only weak expression in 1/3 cases. Comparison of these 3 cases to *EWS-ATF1*-positive GI CCS (previously published and additional unpublished cases from our institution) suggested that GI CCS show less melanocytic differentiation regardless of the fusion type.

Conclusions: The recurrent variant fusion *EWS-CREB1*, representing a t(2;22)(q32;q12), may define a novel subset of CCS that occurs preferentially in the GI tract and shows little or no melanocytic differentiation.

22 Malignant Vascular Tumors of Bone: A Clinicopathologic and Radiographic Study of 78 Cases

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Background: We reviewed 78 cases of malignant vascular tumors collected at the Rizzoli Intitute in order to better understand the pathologic features and how they correlate with biologic behavior.

Design: Of 155 cases coded as malignant vascular tumors, 78 met the criteria for low-grade hemangioendothelioma or high-grade angiosarcoma. Epithelioid hemangioendothelioma was excluded. Histologically, at least focal vasoformative growth pattern was required for inclusion. Tumors were graded on a scale of 1-3 based on the extent of cytologic atypia. Radiographs were available for review on 55 cases. Data on treatment and follow-up was available on 59 pts.

Results: 52 were males and 26 females. Bones involved were: long bones 40, small bones of foot or hand 3, vertebra 5, and flat bones (pelvis, sacrum, rib) 13. 17 pts had radiographic evidence of multicentric disease. Radiographically, a well marginated rim was seen in 16 cases (15 were gr 1 or 2). 39 had an aggressive, purely lytic radiographic appearance with cortical disruption (15 = gr1or 2; 24 = gr 3). Histologically, lower grade tumors were more vasoformative than higher grade tumors. Tumor cells were round to oval shaped with occasional spindling in solid areas. Gr 1 tumors had minimal cytologic atypia. Nuclei in gr 2 tumors were generally uniform, but larger and with moderate hyperchromatism. Gr 3 tumors contained prominent nuclear pleomorphism. Results of grading: gr 1= 20; gr 2=22; gr 3=36. Surgical treatment included: 16 curettage (gr 1=9; gr 2=2; gr 3=5); 16 wide resection (gr 1=5; gr 2= 6; gr 3=5); 13 amputation (gr 1=0; gr 2=4; gr.3=9). 10 pts had a biopsy followed by radiation (gr 1=2; gr 2=2; gr 3=6). Follow-up information (59 pts) ranged from 1 to 216 mos; ave 46 mos. Five died of unrelated causes; 27 were alive w/o disease and 27 DOD (gr 1=0; gr 2=6; gr 3=21). One pt died of a post radiation sarcoma and another is alive with stable lung metastases. Treatment of pts who DOD included: biopsy with radiation: 8 (gr.2=2; gr.3=6); curettage: 5 (all gr. 3); wide margin: 6 (gr 2:=2; gr 3=4); amputation: 8 (gr 2=2; gr. 3=6).

Conclusions: The biologic behavior of malignant vascular tumors of bone is related to the histologic grade. Gr 1 tumors behave in a relatively benign fashion. All of the 16 gr 1 tumors in this series are alive following conservative treatment. Gr 3 tumors are highly aggressive; 21 (of 25) with treatment and follow-up information DOD. Gr 2 tumors show histologic features and biologic behavior in-between gr 1 and gr 3 tumors; 6 (of 16) DOD.

23 Immunohistochemistry and FISH on Tissue Microarrays in the Evaluation of Inflammatory Myofibroblastic Tumor

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Background: Various gene fusions involving the anaplastic lymphoma kinase (ALK) gene at chromosome 2p23 have been described in inflammatory myofibroblastic tumor (IMT), suggesting a neoplastic etiology. The ALK rearrangement is reportedly more common in children; there are only rare IMTs in adults with genetic information. This study represents the largest series of lesions designated as IMT on morphologic grounds, with comparative ALK IHC and FISH analysis.

Design: 16 IMTs were retrieved from the UNM and UVa archives. Inclusion criteria required spindle cells, inflammatory cells, and thorough clinical, histological and IHC exclusion of other diagnostic possibilities. *IHC:* Monoclonal ALK-1 Ab was applied, with evaluation of nuclear and cytoplasmic stain intensity and distribution. *FISH protocol:* 2mm tissue cores were assembled into a tissue microarray (TMA). The ALK breakapart genomic marker was applied to TMA sections. MetaSystems automated FISH analyzer detected signal patterns on interphase nuclei, with results interpreted on a Meta workstation.

Results: Pts aged from 2-70 yrs. (median 48; mean 43). IHC and FISH were successful on 16/16 and 10/16 cases, respectively.

ALK IHC and FISH

	Cervix (1)	Lung (5)	Bladder (2)	Soft Tissue (5)	Breast (1)	Liver (1)	Esophagus (1)
ALK IHC cytoplasm	1/1 3+D	0/5	1/2 3+D	0/5	0/1	0/1	0/1
ALK IHC nuclear	1/1 3+F	0/5	0/2	0/5	R 1+	0/1	0/1
ALK FISH status	Positive	Negative	Neg (1); ND (1)	Neg (3); ND(2)	ND	ND	ND

D=diffuse; F=focal; R=rare; ND=no data

Conclusions: 1) ALK overexpression (IHC) and genomic rearrangements (FISH) are uncommon in our series of IMTs. This may be related to the older age of the patients in this study, compared to previous reports. Furthermore, previous studies preselected only IHC ALK positive cases for genetic evaluation, whereas we included all cases which met the morphologic criteria for IMT. 2) ALK IHC negative cases do not show ALK gene rearrangement by FISH. Our one positive case by FISH correlated with strong ALK IHC reactivity; interestingly, this case showed aggressive clinical behavior. On the basis of these findings, it is clear that the designation "IMT" is an umbrella term in which ALK positivity, at the IHC or genetic level, cannot reliably be used for diagnostic purposes. Further investigation is warranted to determine if ALK positive lesions represent a different, more biologically aggressive entity, necessitating a distinct designation.

24 Immunohistochemistry for Beta-Catenin Is Specific, but Not Sensitive, in the Diagnosis of Desmoid Fibromatosis

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Background: Nuclear staining of beta-catenin by immunohistochemistry is being increasingly used to diagnose desmoid tumors (deep fibromatoses), especially where the differential diagnosis includes other fibrosing, spindle cell neoplasms of the abdomen. The specificity for desmoid fibromatosis is believed to result from the beta-catenin mutations that play a role in the sporadic lesions, and which result in activation of the Wnt signaling pathway.

Design: Beta-catenin expression was evaluated by immunohistochemistry in a total of 284 soft tissue tumors. The cases included 44 desmoid tumors (including 13 in patients with familial adenomatous polyposis), 25 superficial fibromatoses, 19 gastrointestinal stromal tumors, 21 leiomyosarcomas (including 7 intra-abdominal), 25 schwannomas, 26 neurofibromas, 25 solitary fibrous tumors, 10 low grade myofibroblastic sarcomas, 19 desmoplastic fibroblastomas, 21 cases of nodular fasciitis, 11 fibromas of tendon sheath, 1 fibromatosis colli, 5 Gardner fibromas, 15 inflammatory myofibroblastic tumors, 5 infantile fibrosarcomas, 5 lipofibromatoses, and 4 calcifying aponeurotic fibromas. Nuclear and cytoplasmic staining were evaluated separately.

Results: Positive nuclear staining was detected in 48% of cases of sporadic desmoid fibromatosis (15/31), but surprisingly in only 15% of cases in patients with FAP (2/13). No nuclear staining for beta-catenin was present in the schwannomas, neurofibromas, solitary fibrous tumors, superficial fibromatoses, desmoplastic fibroblastomas, nodular fasciitis, inflammatory myofibroblastic tumors, low grade myofibroblastic sarcomas, fibromas of tendon sheath, fibromatosis colli, Gardner fibromas, infantile fibrosarcomas, lipofibromatoses, or calcifying aponeurotic fibromas. Numerous tumors showed localized, dot-like cytoplasmic staining, which can histologically mimic nuclear staining and make interpretation difficult. This cytoplasmic staining was nonspecific, and was present in the majority of tumor types examined, including desmoid fibromatoses.

Conclusions: Nuclear staining for beta-catenin, when present, is specific in the diagnosis of desmoid fibromatosis. In our series, however, only 48% of sporadic desmoid fibromatoses and 15% of desmoid fibromatoses in patients with FAP were positive for the marker, making interpretation of a negative result less helpful and demonstrating that the marker has low sensitivity. Localized, dot-like cytoplasmic staining makes interpretation extremely difficult, and can mimic nuclear staining.

25 Comparison of Solitary Fibrous Tumors and Hemangiopericytomas by Gene Microarray Analysis

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Background: Solitary fibrous tumor (SFT) and hemangiopericytoma (HPC) represent groups of apparently closely-related soft tissue neoplasms which both exhibit a wide clinical and histologic spectrum. The genetic pathogenesis of these groups of tumors remains largely unknown, as well as their relationship at a genetic level.

Design: We examine 16 tumors classified as SFTs with both gene microarrays and array comparative genomic hybridization (aCGH), and compare these results to gene microarray analysis of 4 cases of HPC. We also look for gene expression and chromosomal alterations that may play a central role in the tumorigenesis of these neoplasms, and compare genes which are upregulated in SFTs versus HPCs.

Results: We identified multiple genes which are upregulated in SFTs versus HPCs. By aCGH, we found chromosome 8 amplification in multiple SFTs, and correlate this with gene amplification by mRNA. We found a number of genes to be significantly elevated on chromosome 8. We determined that SFTs are indeed a cohesive group of tumors at the genetic level. Two of the HPCs in our study clustered with SFTs, while the other 2 clustered with separate tumors, specifically gastrointestinal stromal tumors, synovial sarcomas and dermatofibrosarcoma protuberans. While 2 of the HPCs showed some overlap with SFTs, they clustered with the atypical/malignant SFTs and not the typical SFTs. Genes highly expressed in the HPCs but not SFTs included ras, myc and fos family members, while the SFTs highly expressed many genes including collagen XIa, insulin-like growth factor receptor, and wnt2.

Conclusions: These results, while preliminary, show that SFTs form a cohesive group of tumors with reproducible genetic alterations, despite the variety of histologic presentations and locations in which they occur. In addition, the results suggest that while some cases of HPC are closely related to SFTs, other cases diagnosed as "HPC" likely represent other tumors which merely show histologic features mimicking HPC, but are characterized by genetic changes of other tumor types.

26 Gene Expression Analysis of Mixed Liposarcoma

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Background: Liposarcomas of mixed type are rare. We experienced a case of liposarcoma composed of pelomorphic and well differentiated components.

Design: To identify molecular alterations in this tumor, gene expression in pleomorphic liposarcoma (PL), well differentiated liposarcoma (WDL), and normal adipose tissue (NA) was examined using cDNA microarray representing 17,000 genes.

Results: The cDNA microarray analysis showed that 6 genes in WDL, not in PL, were upregulated more than 5-fold compared to NA, and 40 genes in PL, not in WD, were upregulated. Other 6 genes in both WDL and PL, were downregulated more than 5-fold compared to NA. The differentially expressed genes included those associated with signal transduction, transcription, cell cycle, enzyme, structural protein, immune system and others.

Conclusions: Our experimental data domonstrated that multiple genes are differentially expressed in liposarcoma of mixed type. It is suggested that these genes are involved with the differences in morphological characteristics and carcinogenesis of liposarcoma.

27 Gardner Fibroma (GAF): A Clinicopathologic and Immunohistochemical Analysis of 42 Patients

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Background: GAF is a benign soft tissue lesion with a predilection for childhood and adolescence and an association with familial adenomatous polyposis (FAP) and desmoid-type fibromatosis (DES). We report 42 patients with GAF with clinicopathologic correlation and immunohistochemical analysis for beta-catenin (BCAT) and related proteins.

Design: 42 patients with 52 GAFs were identified from surgical pathology and consultation files. Clinicopathologic information included family history of intestinal polyps, colon cancer, and soft tissue tumors. Immunohistochemistry for BCAT, cyclin-D1, and C-myc expression was performed.

Results: 42 patients had 52 GAFs. Information about family history, intestinal polyps, colon cancer, and soft tissue tumors was available in 22 patients: 73% had known APC, 14% had no history of familial polyps or soft tissue tumors, and 14% had an individual or family history of soft tissue masses/DES, with followup periods of 1-16 years. The age range at initial diagnosis was 2 months to 24 years; 79% were diagnosed in the first decade, 16% in the second decade, and 5% in the third decade. Eight patients (19%) had documented DES concurrently or later (6 with APC, 1 with familial DES). Sites included back/paraspinal region (62%), head/neck (15%), extremities (11%), and chest/abdomen (12%). All displayed a hypocellular proliferation of haphazardly arranged coarse collagen fibers with bland spindle cells, small blood vessels, and a sparse mast cell infiltrate. Immunohistochemically, 75% showed nuclear reactivity for BCAT (all with APC) and 100% showed nuclear reactivity for both cyclin-D1 and C-myc. BCAT reactivity had no correlation with age, or site, or recurrence; 2 BCAT-negative GAFs were from APC patients.

Conclusions: GAF has a predilection for childhood and early adulthood, a strong association with adenomatous polyposis coli, an association with concurrent or subsequent development of DES, and over-expression of beta-catenin and other proteins in the APC and WNT pathways. The proportion of sporadic GAFs that are associated with DES and APC mutation is not yet known. This is a significant question for future clinical management and BCAT immunohistochemistry may prove to be useful in addition to tests for APC mutation.

28 Inflammatory Myofibroblastic Tumor (IMT): Comparison of Clinicopathologic, Histologic, Immunohistochemical Features, and ALK Expression in Atypical and Aggressive Cases

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Background: IMT is a neoplasm of intermediate biologic potential. We studied a subset of IMTs with histologic atypia and/or clinical aggressiveness for clinicopathologic features, outcome, immunohistochemical expression of ALK-1, proliferative, tumor suppressor, apoptotic, and prognostic markers to identify potential pathologic-prognostic features.

Design: 59 IMTs with classic, atypical histologic features (cellularity, ganglion-like cells, round/large cells, necrosis), local recurrence, metastasis, were studied. Immunohistochemistry was performed for ALK-1, proliferation (Mib-1), apoptosis (C-myc, cyclin-D3, caspase-3, bcl-2, MCL-1), and prognostic markers (survivin, p27, CD56, p53, MDM-2).

Results: The 5 classic, 21 atypical, 27 locally recurrent, and 6 metastatic IMTs had an age at diagnosis from 3 wk to 74 yr (mean 13.2 yr, 44% in 1st, 27% in 2nd, 29% in later decades). Mean tumor size was 7.8 cm. Sites included abdomen/pelvis (A/P, 64%), lung (22%), head/neck (8%), extremities (5%). Followup ranged from 3 mo to 11 yr. A/P IMTs had a higher recurrence rate (85%); recurrent and metastatic IMTs were larger. Cytoplasmic ALK-1 reactivity was seen in 55% (78% atypical, 52% recurrent, 60% classic, 0 metastatic). ALK-neg. IMTs occurred in older patients (mean age 20.1 y) and had greater nuclear pleomorphism, atypia, and atypical mitoses. > 90% expressed p53, C-myc, cyclin-D1, MDM-2, MCL-1, p27, caspase-3, and survivin, without significant differences among subgroups. None expressed bcl-2 or CD56. Among 27 recurrent IMTs, 10 had no evidence of further disease after resection, 13 had multiple local recurrences, 5 died from local disease (4 ALK-neg.), and 1 had histologic malignant transformation. All six IMTs with distant metastases (lung, mediastinum, brain) were ALK-neg.
Conclusions: Among 59 IMTs, ALK reactivity was associated with local recurrence but not distant metastasis, which was confined to ALK-neg. lesions. Absent ALK expression was associated with a higher mean age, subtle histologic differences, and death from local disease (4 of 5 recurrent IMTs). Other proliferative, apoptotic, and prognostic markers displayed similar patterns in all subtypes of IMT and did not predict behavior. In conclusion, ALK reactivity may be a favorable prognostic indicator in IMT, and A/P IMTs recur more frequently.

29 CTSP-1, a New Cancer-Testis Antigen Are Expressed in Sarcomas, but Not in Benign Mesenchymal Tumors

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Background: Cancer/Testis (CT) antigens are immunogenic proteins expressed in normal gametogenic tissues and in different types of tumors. CT antigens are promising candidates for cancer immunotherapy (CIMTx) and the identification of novel CT antigens is a pre-requisite for the development of polyvalent cancer vaccines. Sarcomas account for approximately 1% of adult malignancies, and may pose a significant diagnostic and therapeutic challenge. Sarcomas represent a great opportunity for antigen discovery because in some cases they have a specific gene translocation that may provide unique antigenic epitopes or regulate expression of normally silenced genes. Besides that, current therapeutic options in the treatment of these tumors are limited. The expression of several CT antigens has already been reported in many types of sarcomas. Previously, we have identified a novel CT antigen, named CTSP-1, which has a very restricted expression pattern among normal tissues. CTSP-1 is exclusively expressed in normal testis and is aberrantly expressed in 44.4% (75/169) of tumors from different primary sites. The highest percentages of positive expression were observed in melanomas (59%) and prostate adenocarcinomas (58%). Anti-CTSP-1 antibodies were detected in 19.8% (28/141) of plasma samples from patients with a wide spectrum of tumors.
Design: We analyzed CTSP-1 mRNA expression by RT-PCR in 74 cases of mesenchymal tumors including 14 cases of benign tumors (10 fibromatosis, 1 leiomyoma, 2 neurofibromas and 1 schwannoma) and 60 cases of sarcomas (8 fibrosarcomas, 14 leiomyosarcomas, 1 GIST, 2 alveolar soft part sarcomas, 4 liposarcomas, 7 MPNST, 13 pleomorphic sarcomas and 11 synovial sarcomas).
Results: Out of the 74 mesenchymal tumors analyzed, 12 express CTSP-1 mRNA. Interestingly, CTSP-1 mRNA expression was not detected in any benign mesenchymal tumor. Among the sarcomas we observed CTSP-1 mRNA expression in 6/14 leiomyosarcomas, 4/13 pleomorphic sarcomas, 1/8 fibrosarcomas and 1/11 synovial sarcomas. We have not observed expression in MPNST, GIST, Alveolar soft part sarcoma and lipossarcoma cases.
Conclusions: In conclusion, CTSP-1 mRNA is only express in malignant mesenchymal tumors, with emphasis in leiomyosarcomas. To our knowledge, CTSP-1 is the most frequently expressed and immunogenic CT antigen in malignant tumors described so far and due to its very restricted expression pattern in normal tissues should be considered as a promising candidate for CIMTx.

30 Translocation and Expression of *CSF1* in Pigmented Villonodular Synovitis, Tenosynovial Giant Cell Tumors, and Reactive Synovial Lesions

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Background: Tenosynovial giant cell tumor (TGCT) and pigmented villonodular synovitis (PVNS) are related conditions with features of both reactive inflammatory disorders and clonal neoplastic proliferations. We recently demonstrated that *CSF1* (1q13), the ligand of a tyrosine kinase receptor, *CSF1R*, can be translocated in TGCT and PVNS; and that a minority of these cases involve fusion to *COL6A3* (2q37). Tumors bearing this translocation may be amenable to treatment with a small molecule inhibitor of *CSF1R*.
Design: We collected 89 cases of TGCT, PVNS, and other synovial lesions and examined *CSF1* and *CSF1R* expression by chromogenic RNA in situ hybridization and *CSF1* translocation by fluorescence in situ hybridization. These studies were correlated with a variety of histologic features. These findings were then compared against the diagnoses as determined by an expert in soft tissue tumor pathology.
Results: We identify three groups of PVNS and TGCT cases by the presence of *CSF1* translocation and *CSF1* expression. The first group (35 cases) had the *CSF1* translocation and also demonstrated high expression of *CSF1* RNA. The second group (16 cases) had high expression of *CSF1* but did not have the translocation as detected by FISH. The third group (8 cases) had neither the translocation nor high *CSF1* expression. Sheets of mononuclear cells, degree of fibrosis and the presence of giant cells correlated with the presence of the *CSF1* translocation. In addition, we noted a striking pattern of *CSF1* expression in cases of reactive synovium.
Conclusions: The translocation and expression of *CSF1* is heterogeneous in TGCT and PVNS. We find some trends in the histology of the tumors associated with the *CSF1* translocation and *CSF1* expression. The *CSF1* expression pattern in reactive synovitis suggests that *CSF1* may play an important role in the inflammatory response of synovium. These findings suggest that small molecule inhibitors to CSF1R may be effective in the treatment of TGCT and PVNS and inflammatory joint disease.

31 Intraosseous Benign Notochord Cell Tumors (BNCT): Further Evidence Supporting a Relationship to Chordoma

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Background: Previous studies have documented the existence of intraosseous benign notochordal cell tumors (BNCT) within the axial skeleton. Evidence suggests that they may be associated with the development of chordomas. To further explore this relationship we reviewed the histologic slides of a series of sacrectomy specimens excised for primary chordoma in search of coexisting BNCTs.
Design: The study group consisted of 82 sacral chordomas resected between the years 1983 and 2005. Available H and E slides were reviewed to identify BNCTs and assess their relationship with the co-existing chordoma. BNCTs were defined, in accordance with prior descriptions, as cohesive aggregates of large cells that appeared adipocyte-like because of their vacuolated cytoplasm. The cells exhibited only minimal nuclear atypia and lacked lobulation and myxoid stroma.
Results: We identified 7 BNCTs, each was adjacent to but separate from the sacral chordoma and a single L4 lesion. The patients ranged in age from 40 to 70 years (mean 58 years) and six were females and two males. Five lesions arose in the sacrum.Two lesions arose in the coccyx, one of which involved two contiguous vertebral levels. The BNCTs ranged in size from 1 to 20 mm with a mean size of 6.1 mm. The lesions were exclusively composed of adipocyte-like nuclei without significant nuclear atypia or myxoid stroma. Four lesions contained sclerotic bony trabeculae and intralesional hematopoietic elements were identified in two cases. In all cases the chordoma was of the conventional type and were morphologically different from the BNCT.
Conclusions: BNCTs were identified in 8.5% of sacral resections performed for primary chordoma. We speculate that this finding provides further evidence that BNCT is the precursor lesion for chordoma. Additional investigations are needed to further understand this relationship.

32 Sarcomas Arising in Paget Disease of Bone, Still Bad after All These Years: A Clinicopathologic Analysis of 68 Cases

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Background: Sarcomas arising in the setting of Paget's disease (PD) of bone are rare, aggressive tumors with a poor prognosis. Since the advent of modern chemotherapy (1985), prognosis in conventional osteosarcomas has greatly improved; however, this has not been reported in osteosarcomas arising in PD. We present 68 cases of PD sarcoma, diagnosed from 1920 to 2005, with clinicopathologic correlation.
Design: We reviewed 68 cases of PD sarcoma and evaluated selected histologic (grade, tumor type) and clinical features (age, gender, site, therapy, duration of PD, tumor size).
Results: Sixty-eight cases of sarcoma arising in PD were identified (34 cases previously reported). Tumors occurred primarily in men (47M:21F), affected older patients (31 - 88 years, mean 66 years, median 68 years) and were located in the pelvis (24), humerus (13), femur (9), calvarium (5), tibia (4) and other sites (11). The incidence was similar in monostotic and polyostic disease. In 35 patients, the diagnosis of PD was made at the time of sarcoma diagnosis whereas in 24 patients, a history of PD from 1 to 30 years was elicited (median 10 years, mean 15 years). The majority (90%) of tumors were osteosarcomas (61/68) with 37 (61%) osteoblastic osteosarcomas, 19 (31%), fibroblastic osteosarcomas and 5 (8%) chondroblastic osteosarcomas. In addition, malignant fibrous histiocytoma of bone (4), fibrosarcoma (2), and giant cell tumor of bone (1) were seen. Follow-up information was obtained in 67 cases (range 1 to 252 months);1 case was lost to follow-up. Survival ranged from 1 month to 31 years (5-year survival of 13%). Of patients for whom cause of death was known (47), 87 % died of disease (range 1 month to 228 months, mean 20 months, median 12 months). Three patients were alive without disease at 3 months and 20 and 21 years, respectively. Median survival for patients diagnosed with PD osteosarcoma before 1985 and after 1985 was 10 months and 16 months, respectively.
Conclusions: Contrary to previous evidence, there is no significant difference in the incidence of PD sarcomas arising in monostotic and polyostotic disease. In approximately one-half of patients, the presence of PD is unknown at the time of diagnosis. Despite improved adjuvant therapy, prognosis in PD sarcomas remains dismal.

33 Prognostic Indicators in Cutaneous Angiosarcoma: Are There Any?

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Background: Histologic grading of soft tissue sarcomas, as defined by tumor differentiation, necrosis and mitotic activity, is valuable in determining prognosis in most tumor subtypes. However, angiosarcomas have traditionally been excluded from grading systems. We have studied a large series of cutaneous angiosarcomas in order to determine what clinical and histologic features may be useful in prognostication.
Design: We reviewed 60 cases of cutaneous angiosarcoma, not associated with previous irradiation or lymphedema. Selected histologic (nuclear grade, presence of necrosis, vasoformative vs solid architecture, chronic inflammation and epithelioid morphology) and clinical features (age, gender, site, tumor depth, invasion and size) were evaluated.
Results: Angiosarcomas occurred in adults (21-94 years, median 71, mean 69), with a male predominance (35M:25F). Tumors occurred in the head and neck (45), trunk (4) and extremities (11) and ranged in size from 0.3 to 15 cm. Eight patients had multifocal

disease. Necrosis was seen in 11 (18%) and epithelioid morphology in 17 (28%). Four tumors had low nuclear grade (7%). Patients were treated with surgery (12), surgery plus chemotherapy and/or radiation (19), and chemotherapy and/or radiation (10). Three patients were treated palliatively and, in 16 patients, the form of therapy was unknown. Follow-up information was obtained for all patients: 40 patients had died (18 of disease, 5 of other causes and 17 of unknown cause) and 20 patients were living (range 9-158 months, median 33 months, mean 65 months). By univariate analysis, older age (p<.0001), multifocality (p=0.037) and epithelioid morphology (p=0.0087) were associated with increased mortality; no other criterion was significant. Location in the head and neck region was associated with increased 5-year survival. By multivariable analysis, male gender (p=0.045), older age (p=0.003) and the presence of necrosis (p=0.0066) were associated with increased mortality. Tumor size correlated with risk of metastasis (p=0.0068) and tumor depth with risk of recurrence (p=0.032).
Conclusions: Histologic features traditionally associated with conventional grading systems are poorly predictive of outcome in cutaneous angiosarcomas. It is possible that a weighted combination of clinical (patient age and gender and tumor focality and site) and histologic (epithelioid morphology and necrosis) features may allow better risk stratification in these patients.

34 Analysis of Protein Expression of c-kit and Gene Mutation of *c-kit* and *PDGFR* in Ewing Sarcomas

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Background: Ewing sarcoma shows 5-year survival rates of only 50% despite the use of multimodal therapeutic approaches. Search for new therapeutic targets and development of novel therapeutic modalities are therefore needed. Activating mutation in *c-kit* and *PDGFRA* gene has been reported as alternative oncogenic events in the pathogenesis of GISTs. Imatinib (Gleevec, Novartis, Basel, Switzerland) is a selective inhibitor of KIT, PDGFR and ABL tyrosine kinase activity and have revealed the anti-tumor effect in CML and GIST. The aim of this study was to evaluate the immunohistochemical expression of the KIT and mutational status of *c-kit and PDGFR* genes in Ewing sarcoma to determine the possible use of imatinib as treatments for Ewing sarcoma.
Design: Methods: In this study, we collected 76 formalin-fixed, paraffin-embedded Ewing sarcomas from Korea, U.S.A., Brazil, and Argentina, and Italy. Immunohistochemistry for KIT was performed using the polyclonal antibody against c-KIT. Mutational analysis was performed by PCR amplification followed by sequence analysis and included exon 9,11,13, and 17 of *c-kit* gene, exon 12 and 18 of *PDGFRA* gene, and exon 12 of *PDGFRB* gene.
Results: Immunohistochemical KIT expression was demonstrated in 30 out of 76 Ewing sarcoma sample (39%). 21 cases (28%) showed strong and diffuse membranous and/or cytoplasmic staining. 9 cases (11%) were diffuse but weakly positive. Activating mutations of KIT were found in 2 out of 76 Ewing sarcoma (2.6%) within exon 9. No activating mutations in the *PDGFRA* and *PDGFB* genes were found except mild pleomorphism in exon 18 of *PDGFRA* gene.
Conclusions: Taken together, immunohistochemical Kit expression was 39 % and activating gene mutation was 2.6 %. There are big discrepancies between immunohistochemical staining versus gene mutation study. Other mechanisms like autocrine/paracrine stimulation of the KIT kinase activity as well as activating gene mutation are probably responsible for KIT expression in Ewing sarcoma.

35 Correlation of Fluorescence In Situ Hybridization (FISH) of Formalin-Fixed Tissue with RT-PCR and/or Cytogenetics in the Detection of Rearrangements of the *FKHR* (13q14) Gene in Alveolar Rhabdomyosarcoma

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Background: Alveolar rhabdomyosarcoma (ARMS) is an aggressive neoplasm with unique recurrent chromosomal translocations present in approximately 80% of cases, with either a t(2;13)(q35;q14) or t(1;13)(p36;q14) resulting in the gene fusions *PAX3-FKHR* and *PAX7-FKHR*, respectively. We report our experience with a dual-color break-apart FISH probe for detecting *FKHR* translocations in formalin-fixed, paraffin-embedded tissue (FFPET) and correlate the findings with reverse transcriptase polymerase chain reaction (RT-PCR) and/or cytogenetics.
Design: FISH for the *FKHR* (13q14) gene rearrangement was performed on FFPET from cases indexed as ARMS (n=10) and embryonal rhabdomyosarcoma (ERMS; n=2). To allow for evaluation of the *FKHR* probe specificity, FFPET from cases indexed as Ewing's sarcoma/Primitive neuroectodermal tumor (EWS/PNET; n=5) with RT-PCR verified *EWS-FLI1* fusion transcripts were included as a negative control. Whole tissue sections were subjected to FISH, blinded to both the diagnosis and RT-PCR data, and a minimum of 100 tumor nuclei per case were scored for the presence of fused (normal) or split (translocated) signals.
Results: All of the ARMS cases (n=10) showed either one of the characteristic cytogenetic abnormalities or harbored one of the two fusion transcripts of ARMS. FISH for the *FKHR* rearrangement in the ARMS cases showed 100% concordance with the RT-PCR and cytogenetic data, with 10/10 ARMS positive for a rearrangement of *FKHR* by FISH (mean 97%+ cells/case; range 97-100%). No *FKHR* rearrangements were detected by FISH in the cases of ERMS or EWS/PNET (mean 2%+cells per case; range 0-4%).
Conclusions: When applied to FFPET, *FKHR* (13q14) FISH showed excellent concordance with RT-PCR and cytogenetics in cases of ARMS. The probe has excellent specificity when applied to its potential mimickers such as EWS/PNET (100%) and has a role in the evaluation of small round cell tumors in distinguishing ARMS from other round cell sarcomas. Future development of FISH probes for *FKHR* partner genes in the ARMS-associated translocations (*PAX3* and *PAX7*), will enable specific determination of translocation subtype and provide further prognostic information.

36 Atypical Lipomatous Tumor, Its Variants, and Its Combined Forms. A Study of 61 Cases with 10-Year Minimum Follow-Up

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Background: Atypical lipomatous tumors (well-differentiated liposarcomas) often contain areas of increased cellularity that do not meet the criteria for a traditional dedifferentiated or pleomorphic liposarcomalike component. Interpretation of these areas has been controversial, and some have advanced the concept of low-grade dedifferentiation or a variant of dedifferentiation in regard to them.
Design: Sixty-one cases of neoplasms composed wholly or in part of atypical lipomatous tumor were reviewed. Minimum follow-up was 10 years.
Results: The cases were divided into four groups based on the findings in the initial excision specimen: conventional atypical lipomatous tumor (n = 15), cellular atypical lipomatous tumor (n = 21), dedifferentiated liposarcoma (n = 24), and atypical lipomatous tumor with a pleomorphic liposarcomalike component (n = 1). The term cellular atypical lipomatous tumor was applied to atypical lipomatous tumors having areas of increased cellularity that when nonlipogenic lacked the 5 mitotic figures per 10 high-power fields (maximal rate) required for a dedifferentiated component and when lipogenic fell short of being truly pleomorphic liposarcomalike. Myxoid regions within this spectrum sometimes had prominent or even plexiform vascularity, creating a resemblance to myxoid liposarcoma especially when interspersed small fat cells were present. The most important prognostic factor was tumor location, as none of the 12 patients with a subcutaneous or intramuscular neoplasm died of tumor. Among the 49 patients with neoplasms of central body sites (mostly retroperitoneum), those with dedifferentiated liposarcoma had significantly shorter survival (median 59 months) than those with cellular (median 142 months) or conventional (median 209 months) atypical lipomatous tumor, whereas there was no statistically significant difference between the latter two categories. Patients with atypical lipomatous tumor (either cellular or conventional) in central body sites had significantly shorter survival if the tumor transformed into dedifferentiated liposarcoma in recurrence, and, conversely, those with central body site dedifferentiated liposarcoma had significantly longer survival if it recurred as atypical lipomatous tumor. Metastasis (seven cases) occurred only when the initial specimen or a recurrence demonstrated dedifferentiated liposarcoma.
Conclusions: Neoplasms within the cellular atypical lipomatous tumor group should not be considered dedifferentiated, and do not contain actual myxoid liposarcoma.

37 EGF-R, c-kit, Her2/neu, and Sex Steroid Receptors in Chordoma

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Background: Complete surgical excision and adjuvant proton radiation therapy offer the best chance of long term survival for chordoma patients, since current chemotherapeutic protocols are ineffective. Isolated reports describing the presence of sex steroid receptors in chordomas suggest that these tumors might respond to hormonal therapy. Expression of EGF-R family members has also been recently reported in chordoma. We examined the expression of a number of proteins involved in various signal transduction pathways that might be exploited as molecular therapeutic targets, potentially offering a new treatment modality for chordoma patients.
Design: 22 cases of chordoma were identified in the surgical pathology files at VUMC. H&E-stained slides were reviewed and diagnostic areas were marked for construction of a tissue microarray (TMA). IHC analysis of the TMA for S-100 protein and cytokeratins confirmed the diagnoses. TMA slides were then stained with antisera directed against estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), Her2/c-*erb*B2, epidermal growth factor receptor (EGF-R), and c-*kit*. Results for Her2 and EGF-R stains were recorded according to standard protocols; c-*kit* stains were graded on a similar four-tiered scale.
Results:

Signaling Protein Expression in Chordoma

	Number of cases	Number positive (%)
ER	22	0 (0%)
PR	22	0 (0%)
AR	21	13 (62%)
Her2	22	0 (0%)
EGF-R	22	15 (68%)
c-*kit*	22	13 (62%)

Over half of the cases expressed AR (mean 80% of nuclei; range 20-100%). Many chordomas were positive for EGF-R. Of the 13 cases positive for c-*kit*, most showed only weak immunoreactivity. No specific nuclear staining was observed for either ER or PR and none of the 22 cases demonstrated membranous staining for Her2. No correlation between staining patterns and site of origin, gender, or histologic subtype (chondroid vs. conventional) was observed.

Intensity of EGF-R and c-*kit* staining in chordoma

	0	1+	2+	3+
EGF-R	7	6	5	4
c-*kit*	9	8	4	1

Conclusions: EGF-R is often overexpressed in chordoma, suggesting that EGF-R inhibitor therapy might benefit some patients. Similarly, the presence of AR raises the possibility that chordoma cells might respond to androgen manipulation therapy. That most cases show no staining or only weak staining for c-*kit* suggests that inhibition of other tyrosine kinases accounts for the tumor response seen in a recent Phase I trial of Imatinib in chordoma. In contrast to EGF-R, none of the cases studied expressed the related growth factor receptor Her2, arguing against the utility of Trastuzumab in these patients.

38 Multifocal Epithelioid Osteoblastoma: A Report of 25 Cases

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Background: Osteoblastomas are benign bone-producing neoplasms. Because of their rarity, and variation in histologic appearance, they pose difficult diagnostic problems. Osteoblastomas are frequently overcalled as osteosarcomas and osteosarcomas are sometimes undercalled as osteoblastomas. A study of a large series of osteoblastomas has emphasized the histological variability in this tumor.

Design: We have reviewed the histological features of 781 osteoblastomas in our files. Based on the growth pattern and cytological features, 25 cases were segregated as having a multifocal growth pattern and epithelioid osteoblasts. Roentgenograms were reviewed in 9 cases. Follow-up information was obtained from the consulting pathologists.

Results: The 25 patients consisted of 17 males and 5 females (no information on 3) ranging in age from 2 to 42. There was a distinct predilection to involve the jawbones (56%). Roentgenograms generally showed well-demarcated mineralized lesions with reactive sclerosis. These tumors grew as multiple foci in marrow spaces without entrapment of trabecular bone. The tumor cells had abundant eosinophilic cytoplasm and eccentric vesicular nuclei with prominent nucleoli. Most of the nodules contain small focal bony trabeculae in the center; some of the nodules contained only epithelioid cells. The lesions were treated in a variety of ways but mostly by curettage. Follow-up information was available in 14 patients; there were no recurrences of metastasis.

Conclusions: This series confirms the histological variability seen in osteoblastoma. The follow-up information available so far supports the notion that epithelioid osteoblasts do not confer aggressive characteristics. Sheets of osteoblasts without matrix formation do not necessarily equate with the diagnosis of osteosarcoma as thought previously.

39 Telomere Biology in Giant Cell Tumor of Bone

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Background: Giant Cell Tumor of Bone (GCTB) is a benign tumor known for its unpredictable clinical behavior of local recurrences and in rare occasions distant metastases. GCTB is composed of uniformly distributed osteoclastic giant cells in a background of mononuclear rounded and spindled-shaped cells. Cytogenetically, telomere associations (TAS) are the most common chromosomal aberrations. TAS in general are nearly exclusively found in high-grade malignancies. GCTB has been defined as a polyclonal tumor, recently a recurrent translocation was reported which suggests a possible role for disturbed telomere maintenance. The aim of this study was to further investigate telomere maintenance in GCTB.

Design: 19 samples from 19 patients were studied. A combination of immunofluorescence and FISH was performed applying antibodies directed against PML and hTERT and telomere-PNA-probes. TRAP assay and telomere length assay was performed for functional detection of telomerase activity and alternative telomere lengthening (ALT).

Results: All samples showed positivity for hTERT-PML immunofluoresence. The giant cells, next to spindle shaped cells, also expressed both markers. The TRAP assay demonstrated a heterogeneous telomerase activity while telomere length assay showed normal telomere lengths indicating negative ALT. Confocal microscopy confirmed co-localization of hTERT with PML in association with telomeres.

Conclusions: GCTB demonstrates remarkable telomere maintenance of activated telomerase and inactivated ALT in the presence of normal telomere lengths. 'Active' hTERT and 'inactive' PML co localize at the end of chromosomes in association with telomeres. These findings strongly suggest that the hTERT-PML aggregates are part of a structural telomere protective capping mechanism rather than of a telomere lengthening mechanism. The telomere maintenance in GCTB could be considered as an important key factor in its pathogenesis.

40 Composite Hemangioendothelioma (CHE): Report of Five Cases Including Congenital Lesions

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Background: CHE is a low-grade malignant vascular tumor showing varying combinations of benign, low-grade malignant, and malignant vascular components. The predominant histologic components are epithelioid hemangioendothelioma (HE) and retiform HE. To my knowledge, there have been only 10 cases of CHE reported in the English literature and its nature and biological behavior remains unknown.

Design: In this study, clinicopathologic and immunohistochemical features of five cases of CHE including congenital lesions are described. One case was ultrastructurally investigated.

Results: The patients were all females with a median age of 33.2 years (range, 8-75 years). All tumors occurred in the dermis and/or subcutis. The tumors arose in the foot or lower leg in 4 patients and one patient had multiple tumors in the left upper extremity. Two patients had congenital tumors in the lower thigh and foot, and upper extremity, respectively. The lesions were usually of several years duration. The size of individual tumors ranged from 1.1 to 6.0 cm. The tumors were composed of a complex admixture of histologic components of various vascular lesions. The predominant components were retiform HE and epithelioid HE, which were each present in all cases. Low-grade angiosarcoma-like or lymphangioma-like areas were observed in two cases each. Areas of spindle cell hemangioma or arteriovenous malformation were identified in one case each. The two congenital cases, which exhibited multiple lesions, had angiosarcoma-like components and an angiomatosis-like growth. One patient was associated with Kasabach-Merritt syndrome. Immunohistochemically, all tumors showed positive staining of at least two endothelial markers (CD31, CD34, factor VIII- related antigen). One case showed diffuse strong D2-40 staining. Ultrastructurally, retiform HE components in one case demonstrated the existence of pericytes. Of four cases with follow up (median duration, 10.5 years), three have recurred locally. To date, none of the patients have developed metastases.

Conclusions: This study confirms that CHE is best regarded as a low-grade malignant vascular tumor. None of the patients have developed metastases. The two congenital cases, which exhibited multiple lesions, showed an angiomatosis-like growth. Congenital CHE may involve also malformation or show anomalous nature. This study expands the concept of CHE by adding congenital cases and provides reappraisal of the HE spectrum.

41 Telomerase Expression in Giant Cell Tumor (GCT) of Bone

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Background: Metastases from GCT are considered rare but rates as high as 15% have been reported. Histologic criteria that predict metastatic behavior do not exist. The neoplastic cells of GCT represent only a small subset of a mixed population of mononuclear and giant cells and are thus difficult to pinpoint on histologic sections. Cell culture experiments that enrich for the neoplastic cells suggest the cells are primitive osteoblast precursors that cytogenetically demonstrate telomeric association. Therefore, telomeric reduction and telomerase activity might promote the transformation of GCT. The osteoblast lineage of the neoplastic cells also raises the question: Might there be analogous mechanisms between metastasis in GCT and metastasis in osteosarcoma since the presence of telomerase in osteosarcoma predicts an unfavorable outcome? Telomerase activity has been studied in cultures derived from GCT, but it is not clear which cells, on histological sections, express the enzyme.

Design: This study was aimed at determining the spectrum of telomerase expression and its prognostic significance, if any, in GCT. Histologic, clinical and radiographic findings (Campanacci grade) were tabulated from 46 patients, 6 of whom (13%) had radiographic evidence of pulmonary metastases. Follow-up ranged from 1 to 52 months. Non-decalcified, paraffin-embedded sections of primary and metastatic GCT were tested with a monoclonal antibody to telomerase reverse transcriptase (ALX-804-504, Alexis, San Diego, CA). Cases were considered positive if specific, nuclear, staining was identified in at least 10% of cells.

Results: Nuclear expression of telomerase was identified in 17 of the 46 primary tumors (37%) and none of the three metastatic tumors (0%). Expression was largely restricted to the plump mononuclear cells and an occasional nucleus in multinucleated cells. Areas rich in spindle cells were uniformly negative for telomerase expression. The presence of metastasis did not correlate significantly with either histologic parameters (including vascular invasion), Campanacci grade or the presence of telomerase expression.

Conclusions: The behavior of GCT remains difficult to predict. Our study is the first to demonstrate the distribution of telomerase expression in the heterogeneous cell population of GCTs. Radiographic and histologic parameters continue to provide little predictive value as to the metastatic potential of these tumors.

42 Multidrug Resistance Activity in Human Osteosarcoma Cell Lines and Reversal of Chemosensitivity

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Background: Resistance to chemotherapy is the major cause of treatment failure in Osteosarcoma (OS) patients. One important mechanism of multidrug resistance (MDR) is mediated by the overexpression of cell membrane transporters such as P-glycoprotein (PGP), MDR associated protein (MRP1) and breast cancer related protein (BCRP), that decreases the intracellular concentration of anticancer drugs by acting as drug efflux pumps.

Design: To identify the relevance of specific drug resistance mechanisms in OS, four established human OS cell lines (MNNG, 143B, U2OS and Saos-2) were treated with doxorubicin (DOX), cisplatin (CIS) and methotrexate (MTX) that are the cytotoxic drugs used in the recently started EURAMOS-1 protocol. Dose dependent cell death was analyzed by FACS analysis and MTT assay. PGP, MRP1 and BCRP mRNA expression were determined by real time PCR. To further assess the functional capacity of MDR transporters we measured the uptake and efflux of ^{99m}Tc labelled cationic lipophilic compounds: Sestamibi (MIBI) and Tetrofosmin (TFS). The efficacy of resistance MDR inhibitors, Cyclosporin A (CsA) and Gleevec was evaluated for its ability to reverse the resistance in cell lines. MIBI and TFSwere used as surrogate markers to assess the effective inhibition of MDR transporters.

Results: The OS cell lines showed a broad spectrum of chemosensitivity: U2OS is highly resistant to DOX, CIS and MTX; MNNG is resistant to DOX and MTX and sensitive to CIS; 143B and Saos-2 cells have similar sensitivity to all drugs. Co-incubation with CsA restores the chemosensitivity to DOX and CIS in resistant cell lines, whereas Gleevec sensitized MNNG to MTX. As expected, no significant modulation effect was observed on drug sensitive cell lines. mRNA expression of MRP1 and BCRP was high in MNNG and U2OS cells. Lower uptake and higher efflux rates of MIBI and TFS were observed in resistant cell lines. Co-incubation with CsA enhanced radiotracers accumulation and retention.

Conclusions: We conclude that overexpression of MRP1 and BCRP play an important role in drug resistance in OS. Reversal of chemosensitivity in OS cell lines can be achieved by co-administration of MDR modulators with anticancer drugs. Functional activity of MDR proteins and pharmacological inhibition can be monitored with MIBI and TFS and used as a prognostic factor in OS.

43 Immunohistochemical Expression of Estrogen Receptors in Chondroid Neoplasms

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Background: Chrondrosarcomas (CS) have been shown to express mRNA and nuclear protein of the estrogen receptor (ER) as well as aromatase activity (Modern Pathology 18:12A, 2005), raising the possibility of estrogen-targeted therapy. We studied the immunohistochemical expression of ERs in benign and malignant cartilagenous tumors to correlate the extent and intensity of ER staining with CS grade, type, and presence of dedifferentiation.

Design: Thirty-nine CS (including 5 dedifferentiated and 4 myxoid), 10 enchondromas (EN), and 4 normal cartilage controls (2 males and 2 females) were studied. Following microwave antigen retrieval immunohistochemical staining was performed using Ventana monoclonal anti-ER (clone 6F11), purchased as a prediluted antibody, using the Ventana Es autostainer. Staining was evaluated semiquantitatively according to percent of cells staining (0%, ≤5%, ≤25%, ≤50%, ≥50%, ≥75%, 100%) and intensity of staining on 3 point scale (+/++/+++).

Results: 62% of CS and 60% of EN demonstrated positive staining for ER. Males demonstrated positive staining for ER in 66% of CS and 63% of EN; females demonstrated positive staining for ER in 60% of CS and 57% of EN. Lower grade CS (grade I and II) expressed ER more frequently than did grade III CS; ER was not expressed in dedifferentiated CS. Myxoid CS consistently expressed ER.

Estrogen Receptor Immunoperoxidase Staining in Chondroid Neoplasms

Tumor	N	Total + for ER	>5%	++/+++
CS Grade I	62%	62%	38%	50%
CS Grade II	75%	75%	50%	66%
CS Grade III	25%	25%	0	0
CS Dedifferentiated	0	0	0	0
Myxoid CS	100%	100%	100%	75%
EN	60%	60%	40%	66%

Conclusions: •CS and EN demonstrated similar overall positivity for ER.
•ER staining was present with similar frequency in both sexes in CS and EN.
•ER expression is diminished in high grade CS and lost in dedifferentiated CS.
•Myxoid CS express ER, which might be useful in confirming a diagnosis.
While presence of ER in tumors does not necessarily imply responsiveness to anti-estrogen therapy, it may worthwhile to consider a clinical trial of such therapy in patients with metastatic CS unresponsive to conventional treatments.

44 D2-40 as a Novel Chondroid Marker Differentiating True Chondroid Tumors from Chordomas

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Background: Chordomas and low grade chondrosarcomas both frequently involve the skull base and distal axial skeleton and also share many histologic features, commonalities that can create diagnostic difficulty. Additionally, both chordomas and chondrosarcomas stain positively for S100, while only chordomas typically express cytokeratins. Consequently, positive or negative cytokeratin staining constitutes the only immunohistochemical determinant for differentiating these two entities. A marker that is reliably positive in chondrosarcomas and negative in chordomas has, to date, not been reported. D2-40 is a monoclonal antibody initially developed against M2A, now known as podoplanin, which reacts with a variety of benign and malignant tissues. We have recently observed strong D2-40 reactivity in cartilage and cartilaginous tumors but not in chordomas. In this study, we systemically investigated D2-40 immunoreactivity in a series of chordomas, chondrosarcomas, and enchondromas, in conjunction with cytokeratin and S100 immunostaining.

Design: Paraffin sections of 22 chordomas, 21 chondrosarcomas, and 12 enchondromas were immunostained with D2-40, S100, and PANCK. Stained slides were then evaluated and scored semiquantitatively on the percentage and intensity of immunoreactivity in relevant tissue.

Results: Chordomas were found to express S100 (18/22), and cytokeratins (22/22) but were uniformly not reactive to D2-40 (0/22). By contrast, all enchondromas were diffusely and strongly reactive to D2-40 and antibodies to S100 (both 12/12), and did not express cytokeratins. Chondrosarcomas exhibited more variability in staining, both in intensity and the percentage of the positive tissue. However, much like enchondromas, most chondrosarcomas stained positively with D2-40 (18/21) and antibodies to S100 (19/21), with only one demonstrating focal positive staining for PANCK. Interestingly, 2 of 3 D2-40-negative chondrosarcomas were dedifferentiated and one was metastatic.

Conclusions: When compared to S100, D2-40 is a more specific chondroid marker staining all enchondromas and a majority of chondrosarcomas, but not chordomas. D2-40 may, therefore, be an effective immunohistochemical adjunct to PANCK and S100 in the workup of chondroid-like neoplasms involving the skull base or distal axial skeleton. Dedifferentiated or high grade chondrosarcomas may not stain positively with D2-40. However, the high grade histologic features of these tumors do not resemble those of chordoma, obviating the need for immunohistochemical analysis.

45 Assessment of *MDM2* Amplification Using Fluorescence In Situ Hybridization on Paraffin Embedded Tissues Discriminates Atypical Lipomatous Tumors from Lipomas

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Background: Well-differentiated liposarcoma/atypical lipomatous tumors (WDL/ALT) are cytogenetically characterized by the presence of ring or giant rod chromosomes that contain multiple of copies of the *MDM2* oncogene. Correct histologic discrimination of WDL/ALT from lipomas and pleomorphic lipomas can be challenging on morphologic grounds only. Assessment of *MDM2* amplification using fluorescence in situ hybridization (FISH) was evaluated in a series lipomatous tumors to discriminate WDL/ALT from lipomas.

Design: Twenty-two lipomas, 13 WDL/ALT, and 6 pleomorphic lipomas were evaluated for *MDM2* locus amplification using fluorescence in situ hybridization (FISH) on 4µm paraffin-embedded tissues sections. All experiments were performed by co-hybridizing a *MDM2* custom designed probe for paraffin embedded tissues with a commercially available centromere 12 specific probe [CEP12(D12Z3), Vysis®]. Signal pattern evaluation was performed on 200 cells per tumor specimen without knowledge of the morphologic diagnoses.

Results: All lipomas lacked evidence of *MDM2* amplification, showing only two *MDM2* and two centromere 12 derived signals per cell (*MDM2*/CEP12 ratio=1). All liposarcomas showed evidence of aneuploidy and *MDM2* amplification with usually >50 *MDM2* copies per cell (*MDM2*/CEP12 ratio > 25). In these tumors, *MDM2* amplification was not only observed in the atypical hyperchromatic pleomorphic cells but also in many cells devoid of recognizable cytologic atypia. Pleomorphic lipomas showed a signal pattern consistent with aneuploidy or concomitant gains of both chromosome 12 centromere and *MDM2* locus but without *MDM2* amplification (*MDM2*/CEP12 ratio=1).

Conclusions: FISH for *MDM2* amplification is a robust diagnostic approach to differentiate WDL/ALT from lipomas and pleomorphic lipomas since only WDL/ALT exhibited *MDM2* amplification. *MDM2* amplification was observed not only in the atypical hyperchromatic cells but also in many cells devoid of cytologic atypia, which suggests that *MDM2* amplification may preced the cytologic changes observed in these tumors.

46 Histologic Response to Neoadjuvant Chemotherapy: A Poor Predictor of Outcome in High Grade Extremity Soft Tissue Sarcomas

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Background: To determine the correlation between neoadjuvant chemotherapy-induced pathologic changes with clinical outcome in high grade extremity soft tissue sarcomas (STS).

Design: Patients with common phenotypes of high grade extremity STS without metastasis at diagnosis, treated at a single institution with neoadjuvant chemotherapy (without neoadjuvant radiotherapy) and primary surgical resection between 1994-2003, were identified. The post-treatment tumors were analyzed for histologic response to therapy, including percentages of tumor viability, necrosis, and hyalinization, degree of cellular degeneration, mitotic activity, chronic inflammation, hemosiderin-laden macrophages, and cystic spaces/ hemorrhage. Patients were grouped according to the percentage of viable tumor post-chemotherapy as excellent (≤ 5%), moderate (6-49%), or poor (≥ 50%) responders. Chemotherapeutic response was then correlated with adverse clinical events: metastasis, local recurrence (LR), and death due to disease (DOD).

Results: Thirty-one patients comprised the study set (11 F, 20 M), median age 57 years (range 7-76). The tumors included 16 MFH/ pleomorphic undifferentiated sarcomas, 5 synovial sarcomas, 5 liposarcomas, 3 leiomyosarcomas, 1 fibrosarcoma, and 1 MPNST. Median follow up was 29.5 months (range 6-119.5). Fourteen patients (45%) had an adverse event: 11 (35%) had metastases, 7 (22.6%) had LR, and 5 (16%) had both. Seven (22.6%) are DOD. In this study population, 19% were excellent responders, 10% moderate, and 71% poor. Of the excellent responders, 50% (3/6) developed metastasis (1 DOD); while 66% (2/3) of the moderate and 41% (9/22) of the poor responders had an adverse event. Hyaline change, necrosis, cellular degeneration, chronic inflammation, and mitotic activity similarly did not correlate with clinical outcome.

Conclusions: Histologic assessment of chemotherapeutic response in high grade extremity STS is a poor predictor of clinical outcome.

47 Heat-Shock Protein-90 (HSP-90) Expression in a Spectrum of Benign and Malignant Spindle Cell Neoplasms: An Immunohistochemical Study

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Background: Heat-shock protein-90 (HSP-90) is a cytosolic chaperone molecule that has been shown to be an essential cofactor in the development of many types of cancer. Among the many HSP-90-dependent proteins are transcription factors, cell-cycle regulators such as p53, and the tyrosine kinase family of proteins, including EGFR and HER2. Geldanamycin derivatives are selective inhibitors of HSP-90 that have been shown to destabilize multiple oncogenic tyrosine kinase receptors, prevent proliferation, and induce apoptosis in a variety of malignant neoplasms including sarcomas *in-vitro*. Currently, HSP-90 inhibitors are being studied in Phase II clinical trials for treatment of both hematologic and solid tumors.

Design: Using an HSP-90 specific monoclonal antibody (Labvision Corp), we assessed HSP-90 expression in a tissue microarray consisting of: 64 malignant peripheral nerve sheath tumors (MPNST: 40NF1-related, 24 sporadic), 24 plexiform neurofibromas (PNF), 11 diffuse neurofibromas (DN), 8 localized neurofibromas (LN), 22 schwannomas (S), 4 perineuromas (PN), 12 synovial sarcomas (SS), 5 clear cell sarcomas (CCS), and 8 desmoplastic melanomas (DM). Immunoreactivity was considered positive if greater than 10% of tumor cells expressed positive cytoplasmic staining.

Results: The proportion of HSP-90 positive tumors was similar in both NF1-associated and sporadic MPNST cases, with 17/40 (43%) and 8/24 (33%) demonstrating increased expression, respectively. An even higher percentage of HSP-90 positive cases was observed in the other malignant spindle cell neoplasms. 5/5 (100%) CCS, 8/12 (67%)

SS, and 4/8 (50%) DM demonstrated increased cytoplasmic immunoreactivity. Of the remaining spindle cell lesions, only 1 schwannoma (5%) demonstrated weak cytoplasmic staining, while immunoreactivity was not identified in PNF, DN, LN, or the PN.

Conclusions: HSP-90 is overexpressed in a subset of NF1-related and sporadic MPNST, SS, and DM, and in 100% of CCS examined. With the exception of one schwannoma, no expression of HSP-90 was identified in the benign lesions within this study. Further study of this protein in sarcomas is warranted, as selective HSP-90 inhibitors may be of benefit in their treatment.

48 Epidermal Growth Factor Receptor (EGFR) Expression and Gene Amplification in a Spectrum of Spindle Cell Soft Tissue Neoplasms: A Fluorescence In Situ Hybridization (FISH) and Immunohistochemical (IHC) Study

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Background: EGFR is a transmembrane glycoprotein with tyrosine kinase activity that functions in cell proliferation and survival. EGFR expression and gene amplification have been studied in various tumors. However, EGFR data in soft tissue neoplasms are limited. We assessed EGFR status by FISH and IHC in a variety of benign and malignant spindle cell neoplasms.

Design: EGFR monoclonal antibody (Ventana) and EGFR/CEP7 Dual Color FISH assay (Vysis) were applied to a tissue microarray that included: 62 malignant peripheral nerve sheath tumors (MPNST: 40 NF1-associated, 22 sporadic), 22 plexiform (PNF), 9 diffuse (DN) and 8 localized neurofibromas (LN), 21 schwannomas (SW), 3 perineuromas (PN), 14 synovial sarcomas (SS), 9 DFSP, 5 clear cell sarcomas (CCS), and 9 desmoplastic melanomas (DM). Immunoreactivity was either negative (no staining), 1+ (weak membranous and/or cytoplasmic staining in <10% of tumor cells), 2+ (weak or partial staining in >10% or strong staining in <50% of tumor cells), or 3+ (strong staining in >50% of tumor cells). By FISH, 40 non-overlapping nuclei per tissue core were counted, and EGFR/CEP7 ratio was calculated in each case (EGFR considered amplified if ratio ≥2).

Results: EGFR showed similar 2-3+ expression by IHC in both NF1-associated and sporadic MPNST, with 33/40 (83%) and 17/22 (77%), respectively. Similar expression pattern was also seen in: 16/22 (73%) PNF, 9/9 (100%) DN, 3/8 (38%) LN, 1/21 (5%) SW, 5/9 (56%) DFSP, 3/3 (100%) PN, 13/14 (93%) SS, 2/9 (22%) DM, and 1/5 (20%) CCS. EGFR amplification was identified in 3 MPNST (4.6%; 2 NF1-associated, 1 sporadic; EGFR/CEP7 ratio range: 2.2-4.2) but not in the remaining neoplasms. The 3 FISH-amplified cases demonstrated 3+ IHC reactivity.

Conclusions: Although significant EGFR protein expression was identified in most of the spindle cell neoplasms studied, aside from 3 MPNSTs, gene amplification was not identified. Our findings support the need for further study, as EGFR antagonists may be of benefit to patients with soft tissue tumors that express and are dependent on EGFR.

49 Tumoral Calcinosis-Like Lesions of the Distal Extremities

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Background: Tumoral calcinosis (TC) is a distinctive clinicopathologic entity characterized by soft tissue deposits of calcium near large joints. TC occurs in three clinically and etiologically distinct forms: primary normophosphatemic (PNPTC), primary (hereditable) hyperphosphatemic (PHPTC), and secondary (STC) TC. Herein, we analyze the clinicopathological features of an uncommon subset of TC-like lesions arising in the soft tissue of the distal extremities (TC-L) and evaluate their relationship to conventional TC.

Design: Thirty-two cases of distal extremity soft tissue lesions with histological features similar to TC, but smaller in size, accessioned over a thirty year period were retrieved for clinicopathologic analysis.

Results: There were 21 females and 11 males (Caucasian:non-Caucasian>4:1) ranging in age from 1 to 91 (mean, 39) years. Lesions occurred in fingers (n=16), feet (n=5), hands/toes/wrist (n=4, each), and ankle (n=1); measuring 0.3-3.0 (mean, 1.4) cm. Two patients had separate ipsilateral acral lesions biopsied. Chief initial complaints included presence of a painful (n=8) or asymptomatic (n=7) mass; recent onset of pain/swelling in a preexisting lesion (n=5); and joint immobility (n=1). Factors predisposing to pathological calcification included antecedent trauma (n=5); scleroderma (n=3; TC-L was initial manifestation in 2); rheumatoid/osteoarthritis (n=3); bone deformity (n=3; 2 were congenital); and chronic renal failure (n=2). Histologically, the lesions were mostly deep-seated and consisted of multiple, typically small cystic/cleft-like spaces bordered by histiocytes, osteoclast-like giant cells, and a variable inflammatory infiltrate and containing fibrin, granular calcific debris, and calcospherites. Heavily calcified spaces tended to be less cellular. Fibrocartilaginous tissue (+/- calcification) was noted in 7 lesions. Followup for 18 patients (range: 2 to 30 years; mean:10 years) after local excision showed recurrence/persistence/new lesions in 3 patients (all with scleroderma). No one had family history or developed clinical/biochemical stigmata of PHPTC.

Conclusions: TC-L is histologically similar to conventional TC, but presents as a smaller size lesion. No patient with adequate history/followup data exhibited features of PHPTC. Instead, TC-L is more closely related to trauma-induced PNPTC and STC. TC-L may be the first manifestation of scleroderma, where it has the potential to follow an unrelenting course.

50 GISTs with PDGFRA Exon 14 Mutations Represent Subset of Clinically Favorable Gastric Tumors with Epithelioid Morphology

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Background: Gastrointestinal stromal tumors (GISTs) are common mesenchymal tumors of gastrointestinal tract. Activating KIT or PDGFRA mutations have been shown to be a major force in GIST pathogenesis. Recently, previously undescribed N659K PDGFRA exon 14 mutation has been reported in GISTs. The purpose of this study was to evaluate the frequency of GISTs with PDGFRA exon 14 mutations and define the clinicopathologic profile of such tumors.

Design: GISTs negative for KIT exons 9, 11, 13 and 17 and PDGFRA exons 12 and 18 were evaluated for PDGFRA exon 14 mutation by PCR amplification and direct sequencing.

Results: Mutations were found in 11 of 119 (9%) gastric GISTs. None of 81 GISTs from other than gastric location had such a PDGFRA mutation. Majority of these mutations (8 cases) represented simple 2125C>A or C>G missense mutation leading to substitution of the lysine for asparagine (N659K). However, in two cases 2123A>T missense mutation leading to substitution of the tyrosine for asparagines (N659Y) were found instead. Ten of 11 GISTs with PDGFRA exon 14 mutations had pure epithelioid morphology. One tumor had mixed, predominantly spindle and focally epithelioid cell morphology. Frequency of PDGFRA exon 14 mutations among pure epithelioid GISTs was almost 19%. Immunohistochemically, majority (64%) of these tumors lacked KIT expression or showed only focal scattered KIT positivity. Tumor size ranged from 2.5 to 16 cm (average 7.1 cm). Low mitotic activity, ≤ 5 mitoses/50HPF was detected in 6 GISTs including larger, >5cm tumors. Base on mitotic activity and tumor size, 6 tumors were classified as probably benign with very low malignant potential. Low to moderate malignant potential and high malignant potential was suggested in 3 and 2 tumors respectively. In four cases with moderate or high malignant potential GISTs a long-term follow up (average 235.5 months) showed favorable course of disease.

Conclusions: PDGFRA-MT exon 14 GISTs represent a small subset of gastric tumors with predominantly epithelioid cell morphology. Although almost half of these tumors were classified as expectedly malignant, long term follow-up data suggested a benign course of disease. Therefore, detection of PDGFRA exon 14 mutations may represent additional marker that identifies gastric GISTs with a high probability of benign behavior.

51 Immunohistochemical and Molecular Profiling of Human Synovial Sarcomas Xenotransplanted into Nude Mice. A Tissue Microarray-Based Study*

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Background: Nude mice xenografts of synovial sarcomas (SS) offer an excellent system for the studies of cell heterogeneity and cell differentiation. Our prime objective is to test the utility of tissue microarrays (TMA) as a high-throughput tool in evaluating the histological and immunophenotypic changes between the primary SS and their xenotransplants.

Design: A total of 11 primary SS including 8 monophasic fibrous (MFSS), 2 biphasic (BSS) and 1 poorly differentiated (PDSS) were xenotransplanted into athymic nude mice. Three TMAs were constructed, incorporating all the 11 primary tumors and their xenografts. Immunohistochemistry was performed on TMA sections using 29 markers, which included diagnostic, proliferative, apoptotic, cell cycle and 4 novel markers. The SYT-SSX chimeric transcripts were demosntrated by reverse transcriptase PCR(RT-PCR). Hierarchical cluster analysis was employed in order to group the large TMA immunostaining data.

Results: A transformation of the primary SS to a poorly differentiated phenotype was noted in the xenografts. Immunohistochemically, novel markers such as EGFR and SALL2 were expressed in both the primary tumor and xenografts, therby proving to be specific for SS. ApoD1 and IGFBP2 were negative to sporadically positive. Bcl-2 and bax were enhanced in the xenografts compared to the primary tumors. Increased expression of Ki-67 noted in the xenografts, correlated with high mitotic index. EMA and cytokeratin AE1/AE3 were the most sensitive of the diagnostic markers. RT-PCR detected SYT-SSX transcripts in primary tumors as well as in their passages. Hierarchical clustering differentiated primary MFSS and BSS as well as grouped the antibodies. However, it failed to detect significant immunophenotypic differences between primary tumors and xenografts.

Conclusions: Our study suggests definite genetic instability in the xenografts revealed at the phenotypic level. Immunodetection of EGFR promises to be a potential therapeutic target in SS. Hierarchical cluster analysis is an efficient tool for grouping large TMA immunostaining data. *Supported by a grant (PI-04/0822) from the FIS (Madrid, Spain) and EC (PROTHETS, LSHC-CT-2004-503036) **Holder of a grant from the AECI, Ministry of Foreign Affairs, Spain.

52 Ewing's Sarcoma of Bone: The Detection of Specific Transcripts in a Large, Consecutive Series of Formalin Fixed, Decalcified, Paraffin-Embedded Tissue Samples Using the Reverse Transcriptase-Polymerase Chain Reaction

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Background: The routine use of the RT-PCR technique on decalcified or non-decalcified, formalin fixed, paraffin embedded tissue (FFPET) for tumor-specific translocation detection is hampered by RNA degradation. In order to overcome this difficulty, we

have developed an improved RNA extraction methodology and used new and previously published PCR primers designed to generate small amplicons.
Design: Using RT-PCR to detect specific transcript variants, we prospectively analysed FFPET samples from 54 consecutive cases of Ewing's sarcoma of bone. Samples from approximately half of the cases had undergone formic acid decalcification as part of the routine tissue processing. We also tested the specificity of all the FFPET primers on a series of non-Ewing's sarcoma tumour tissue samples. In 29 of the 54 Ewing's sarcoma cases, corresponding fresh and fresh frozen tissue was available for analysis by one or more of the following techniques: Cytogenetic analysis, RT-PCR and fluorescent in situ hybridisation (FISH).
Results: On the FFPET samples, a Ewing's sarcoma-specific translocation was detected in 52 of the 54 cases (96% sensitivity). Specificity was 100%. Tissue decalcification did not affect the detection rate and in all cases where corresponding fresh/fresh frozen tissue was analysed, the detected translocation type/transcript variant subtype was similar. The relative incidence of Ewing's sarcoma-specific translocation types and transcript variants was entirely consistent with previously published data (table 1).
Conclusions: With equal effectiveness, RT-PCR can be applied to both acid decalcified and non-decalcified FFPET for (Ewing's sarcoma) translocation detection and the technique can be introduced into routine practise in histopathology departments.

Table 1: Summary of detected translocations and transcript variants.

Translocation type	Transcript variant	Number of cases (% of total)
EWS/FLI-1: t(11:22)	7/6 (Type 1)	30 (55.5%)
	7/5 (Type 2)	10 (18.5%)
	10/6 (Type 3)	6 (11.0%)
	10/5	3 (5.5%)
	10/8	1 (2%)
Subtotal EWS/FLI1		50 (92.5%)
EWS/ERG:t(11:22)	7/6	2 (3.5%)
	7/7	1 (2%)
	7/9	1 (2%)
Subtotal (EWS/ERG)		4 (7.5%)
Total		54 (100%)

53 Molecular Detection of FUS-CREB3L2 Fusion Transcripts of Low Grade Fibromyxoid Sarcoma by RT-PCR Using Formalin-Fixed, Paraffin-Embedded Tissues

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Background: The diagnosis of low grade fibromyxoid sarcoma (LGFMS) is challenging due to its bland-looking histological features that are potentially confused with other benign or low grade fibromyxoid lesions such as desmoid-type fibromatoses, perineurioma, and low grade myxofibrosarcoma. Recent cytogenetic and molecular analyses have shown that most LGFMSs have a characteristic chromosomal abnormality, t(7;16)(q33;p11), resulting in the FUS-CREB3L2 fusion gene. However, these assays have been hardly applied to formalin-fixed, paraffin-embedded (FFPE) tumor samples.
Design: FFPE tumor tissues of 17 LGFMSs were retrieved from our archives. RNA extracted from each sample was reverse-transcribed, and synthesized cDNA was analyzed by PCR using primers newly designed by us to amplify specifically most of the junctional regions of the FUS-CREB3L2 fusion gene transcripts previously reported. The primers include three forward primers located on the exon 5 to 6 of the FUS and three reverse primers located on the exon 5 to 6 of the CREB3L2.
Results: The FUS-CREB3L2 fusion gene transcripts were detected in 14/17 (82%) cases of LGFMS. Nucleotide sequence analysis of the PCR products revealed that different portions of the FUS exon 6 or 7 were fused with variable regions of the CREB3L2 exon 5, resulting in 13 patterns of different nucleotide sequences. The FUS-CREB3L2 fusion gene transcripts were not detected by our assay in any case of 123 other soft tissue tumors, including desmoid-type fibromatoses, myxofibrosarcomas, perineuriomas, and congenital or adult fibrosarcomas.
Conclusions: The broad variation of the FUS-CREB3L2 breakpoints, which were detected using FFPE tissues, indicates reliability of the results of the present study. Our method of the molecular assay can be used as a diagnostic adjunct of LGFMS.

54 Cytogenetic Aberrations in Mesenchymal Neoplasms: A Single Institutional Experience

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Background: Many types of soft tissue neoplasms are associated with recurrent cytogenetic abnormalities. The detection of these chromosomal aberrations is diagnostically useful, and their prognostic significance should be elucidated as karyotypes from more cases are reported. To our knowledge, there are no recent published reviews in the English literature of cytogenetic abnormalities in soft tissue tumors from a single institution.
Design: Conventional metaphase cytogenetics was performed in our cytogenetics laboratory on fresh tissue from mesenchymal tumors from our surgical pathology laboratory over a period of four years. Cytogenetics reports, clinical history, and histology were reviewed for 48 soft tissue tumors. The results from an analysis of 34 benign lipomatous tumors performed by our institution were not included in this study (Arch Pathol Lab Med. 2005;129(4):553). Recurrent cytogenetic abnormalities were identified using the Mitelman Database of Chromosome Aberrations in Cancer (2005) and a review of the literature.
Results: 48 cases of benign and malignant soft tissue tumors were reviewed, including 28 distinct morphologies. The patient group included 24 males and 24 females, and patient age ranged from 6 weeks to 85 years. The majority of tumors (75%) were located in the extremities (29%), trunk (23%), head and neck (13%), or retroperitoneum (10%). Cytogenetic abnormalities were observed in 22 (46%) cases, a normal chromosome profile was found in 19 (40%) cases, and 4 (8%) cases had no karyotype reported, due to specimen inadequacy. Several of the cases with normal cytogenetics were dense or fibrous tumors. Of the 22 cases with aberrant karyotypes, 13 (59%) tumors had cytogenetic abnormalities present in two or more cases in the literature. The tumors with recognized aberrations included 4 liposarcomas (1 well-differentiated, 1 myxoid/round cell, 1 dedifferentiated, 1 mixed-type), 2 undifferentiated pleomorphic sarcomas, 2 primitive neuroectodermal tumors, and 1 case each of alveolar rhabdomyosarcoma, high grade myxofibrosarcoma, leiomyosarcoma, malignant peripheral nerve sheath tumor, and lipoblastoma.
Conclusions: Cytogenetic analysis demonstrated abnormal karyotypes in nearly half of this series of mesenchymal neoplasms, the majority of which consisted of recognized aberrations reported in the literature. Our study demonstrates that conventional cytogenetics is practical for a single institution to perform and yields reproducible results. Further, cytogenetics is a useful diagnostic adjunct, especially in difficult soft tissue surgical pathology cases.

55 Sporadic vs Radiation-Associated Angiosarcoma: Biologic and Clinicopathologic Comparisons

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Background: Angiosarcomas arise sporadically or secondary to predisposing conditions such as ionizing radiation, lymphedema or exposure to vinyl chloride. Radiation-associated angiosarcoma (RAA) is rare, usually occurs in the breast, and shows histologic features and poor behavior similar to sporadic angiosarcoma (SA). It is not known whether there are more specific biologic or clinicopathologic differences. To better understand the biology of SA and RAA, we studied biologic and clinicopathologic features of 47 angiosarcomas.
Design: Clinical records and pathology from 47 angiosarcomas were reviewed. Tissue microarrays were immunohistochemically stained for p53, MIB1, hTERT, CD31, and CD34. DNA was sequenced for TP53 and ataxia-telangiectasia (ATM) gene mutations.
Results: Ten (21.3%) patients had prior radiation including six for breast carcinoma. Median latency was 7.1 years (3.2-32.6 years). Six (60%) RAA showed prominent vasoformative growth compared to 10 (27%) SA. Eight (80%) RAA and 27 (86.5%) SA were high grade. Breast RAA tended to involve the dermis (80%) and SA the parenchyma (80%). No RAA overexpressed p53 while 26% of SA did. Proliferative indices were similar in RAA and SA (40% vs. 35% with high MIB1 expression). hTERT was expressed in all angiosarcomas. CD31 and CD34 expression was similar in the 2 groups, with CD31 being more sensitive. Mutation of TP53 was identified in 10% of RAA and 13.5% of SA. No angiosarcomas had ATM mutations. Six (60%) RAA patients died (4 of disease; 40%) compared to and 19 (65.5%) SA (10 of disease; 27%).
Conclusions: We observed higher p53 expression in SA compared to RAA and a more vasoformative pattern in RAA. Histologic grade, immunophenotype, proliferative index and survival were similar. Further studies, especially gene expression profiling, which we are pursuing, may provide more insight into the biology of RAA.

56 Gastrointestinal Stromal Tumors (GISTs) of the Jejunum and Ileum – A Clinicopathologic, Immunohistochemical and Molecular Genetic Study of 906 Cases Prior to Imatinib with Long-Term Follow-Up

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Background: GISTs, the specific KIT- or PDFGRA-signaling driven mesenchymal tumors, are the most common mesenchymal tumors of the GI tract. In this study, a large series of small intestinal GISTs was analyzed to gain better understanding on their frequency, clinicopathologic and molecular genetic features and prognosis.
Design: 1091 tumors originally classified as smooth muscle tumors of the small intestine (excluding duodenum) were analyzed using standard histology, immunohistochemistry and molecular genetics.
Results: 83% of analyzed tumors (906 cases) were diagnosed GISTs.The GIST patients had 55:45 male to female ratio with a median age of 59 years (range, 13-94 years). Only 0.6% of tumors occurred before the age of 21 years and 13.6% before the age of 40 years. The tumors varied from 0.3-40 cm (median 7.0 cm) and most commonly presented with GI bleeding or acute abdomen; 18% were incidentally detected. Histologically the tumors were relatively monotypic with spindle cell (86%), epithelioid (5%), or mixed patterns (9%). Most epithelioid tumors were malignant, and this morphology sometimes emerged from less cellular and less mitotically active spindle cell tumors, suggesting that it represented a transformation. KIT was expressed in 98%, CD34 in 40%, smooth muscle actin in 34%, desmin in 0.2%, and S100 protein in 14% of tested tumors. Outcome was dependent on tumor size and mitotic activity, with an overall 39% tumor-related mortality, twice that for gastric GISTs. Only <3% of tumors ≤5 cm and ≤5 mitoses/50 HPFs metastasized, whereas 86% of tumors > 10 cm and > 5 mitoses/50 HPFs metastasized. In contrast to corresponding gastric tumors, tumors >10cm with mitotic activity ≤ 5/50 HPFs and those ≤ 5 cm with mitoses >5/50 HPFs had a high metastatic rate (>50%); tumors > 5cm ≤ 10 cm with low mitotic rate had 24% metastatic rate. The median survival times of patients with low mitotic rate tumors who died of disease decreased by increasing tumor size. KIT mutations were detected in exon 11 (n= 90), exon 9 (n=17) and exon 17 (n=1); the presence of mutation or mutation type were not prognostically significant. There were no PDGFRA exon 12 or 18 mutations.
Conclusions: Systematic data on prognosis of small intestinal GISTs of various size and mitotic activity categories can be helpful in management and surveillance of patients with these tumors.

57 Fibroosseous Pseudotumor of the Digits (FOPD): 44 New Cases

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Background: Myositis ossificans (MO), a reactive zonal fibroosseous lesion of deep extremity/trunk in young patients, generally matures within weeks to form a final bony rim. FOPD, a similar lesion of superficial digits, is reportedly less organized. FOPD resembles malignant and benign recurrent osteocartilaginous lesions. We wanted to

examine our FOPD from 1980 to present and add to the existing older literature (3 series:1981, 1986, and 2003, 12-21 cases each).
Design: 52 cases (slides, folder, IHC, radiology) coded as "FOPD" or "MO of the hands and feet" were reviewed and analyzed. Those with incomplete material or otherwise diagnosed were excluded.
Results: 44 cases included 18 M and 26 F. Presenting diagnoses: osteosarcoma, chondrosarcoma, fasciitis, giant cell tumor with bone, MO. Radiologic studies: lesions separate from bone. Only 6 patients reported antecedent trauma, but pain/edema and occupational use of hands (i.e. pianist, cashier, automotive mechanic, hull technician) were common. Patient ages: range 10 - 64, mean/median 40 years. Tumor locations: 42 hand /finger and 2 foot/toe; proximal phalanx finger most common. Tumor size: range: 0.2 - 5.0, mean 2.3 and median 1.5 centimeters. All cases were dermal (6 with superficial ulceration) and/or subcutaneous with fasciitis-like stroma (moderate cellularity, mild atypia) and 10-90% (mean 48%) endochrondral (cartilage phase scant and none with cartilage cap) or intramembranous woven bone (wb). The wb was immature (i)/interconnected centrally and mineralized/partial to complete rim peripherally, some later corticallike with fatty marrow replacement; 50% with zonal organization. Grungy calcification (16); adjacent Pacinian corpuscles (8). Rare focal ischemic change (fic). Mitotic activity present, all normal forms. IHC: SMA+, S100 protein -, desmin-, keratins-, additional studies pending. All, except one ray amputation, were locally excised. 3 recurrence/residual FOPD demonstrated maturation.
Conclusions: FOPD is a benign subcutis/dermal osseous and myofibroblastic lesion of the proximal finger in adults. Despite few with trauma history, many patients present with pain/swelling and have manual occupations. Woven bone and fasciitislike components are equally represented; these mature to organization more commonly than previously recognized. Iwb, fic, cellularity, atypia, mitotses, and superficial ulceratin may occur and do not indicate malignancy. Local excision is adequate, most cases do not recur.

58 The Hedgehog Signaling Pathway in Synovial Sarcoma: Mutational and Functional Analysis

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Background: The Hedgehog (HH) pathway signals via Patched (PTCH) and Smoothened (SMO) at the cell surface to GLI1 in the nucleus. It is known to be required for cell differentiation and organ formation during embryogenesis. Deregulation of HH signaling due to mutations in *PTCH* or *SMO* is seen in cases of medulloblastoma (MB) and basal cell carcinoma (BCC). In a previous microarray-based comparison of expression profiles of 46 synovial sarcoma (SS) samples and 91 samples of 4 other translocation-associated sarcomas (Ewing sarcoma, alveolar rhabdomyosarcoma, desmoplastic small round cell tumor, alveolar soft part sarcoma), we observed features of HH pathway activation in SS, specifically *GLI1* and *SMO* were significantly overexpressed, as were some downstream targets of GLI1 such as *WIF1* and *PDGFRA*. This prompted us to perform a mutational and functional analysis of the HH signaling pathway in SS.
Design: Mutational analysis was performed by direct sequencing of all coding exons of *PTCH* and *SMO* (the two genes most frequently mutated in this pathway in MB and BCC) in 40 SS samples and 3 SS cell lines (FUJI, HS-SY-II, SYO-1). We also examined the changes in cell growth and HH target gene expression by blockade of HH signaling using cyclopamine, a specific inhibitor of SMO, in the 3 SS cell lines.
Results: The mutational analysis revealed no somatic mutations in *PTCH* and *SMO* in the 40 SS samples and the 3 SS cell lines. By RT-PCR, all three cell lines expressed *GLI1*, *GLI2*, *GLI3*, *SMO* and *PTCH*. There was a wide range of sensitivity to cyclopamine among three cell lines. FUJI cells were the most sensitive, with an IC50 (50% reduction in cell counts relative to untreated cells at 96 hrs) between 1 μM and 5 μM whereas SYO-1 cells were less sensitive (IC50: 10 μM), and HS-SY-II cells were resistant (IC50>10 μM). We also examined the effect of cyclopamine on genes reported to be transcriptionally up-regulated by HH signaling in other cell types (*GLI1*, *PTCH*, *BCL2*). Quantitative RT-PCR assays showed an 80% decrease in *GLI1* expression relative to untreated cells in the FUJI cells, but not in the other two cell lines. *PTCH* and *BCL2* were not significantly altered upon cyclopamine treatment in any of the three cell lines.
Conclusions: Our data indicate that the HH pathway is functional in some SS, in which it modulates growth and confers sensitivity to cyclopamine, thereby identifying HH signaling as a novel potential therapeutic target. However, mutational activation of the HH pathway is absent or rare in SS.

59 Epithelioid Variant of Myxofibrosarcoma: Expanding The Morphologic Spectrum of Myxofibrosarcoma

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Background: Myxofibrosarcoma (MFS) is one of the most common soft tissue sarcomas of elderly patients and has a predilection for the limbs. Herein, we report an undescribed variant of MFS showing epithelioid morphology.
Design: Sixteen cases diagnosed as epithelioid MFS were retrieved from authors' files from among 570 cases of MFS. H&E stained sections were re-examined and immunostains for pan-keratin (11 cases), S-100 protein (13), desmin (14) and SMA (13) were performed. Clinical details were obtained from referring pathologists and clinicians.
Results: Eight patients were male and 8 female (age range 43-89 yrs; median 67). Fourteen patients presented with a mass, and in 2 there was also pain. Duration of symptoms varied from 1 to 24 months (median 3). Tumor size ranged from 2 to 15 cm (median 6.75). In 9 cases the tumor was located in subcutaneous tissue and in 6 cases it was subfascial. The majority of the tumors were located on the limbs (6 upper and 8 lower) followed by neck (1) and trunk (1). Follow-up was available in 13 patients (range 2-240 months; median 16). Eleven patients were treated by surgery followed by chemotherapy and/or radiation (7 cases). One patient received chemotherapy after incisional biopsy and 1 patient was treated by surgery alone. Nine patients (69.2%) developed local recurrence. Six patients (46.1%) developed metastases to lungs and retroperitoneum. Four patients (30.7%) died of disease so far. Two patients were lost to follow-up. Morphologically, 13 cases were high grade, 2 were intermediate and 1 was low. Tumors were characterized by multinodular, infiltrating growth with alternation of hypercellular and hypocellular myxoid areas; the latter showed prominent curvilinear vessels. Neoplastic cells were arranged singly and in small clusters in the myxoid areas or formed sheets in the hypercellular areas, and showed epithelioid morphology with round nuclei, vesicular chromatin, prominent nucleoli and moderate anounts of eosinophilic cytoplasm. Epithelioid areas were generally multifocal with admixed areas of conventional MFS. Immunostains were negative for all studied markers. Differential diagnosis includes carcinoma, melanoma and myoepithelial carcinoma.
Conclusions: Epithelioid MFS is an unusual variant of MFS with similar clinicopathologic features to its conventional counterpart. Its natural history seems similar to (or more aggressive than) usual high grade MFS with approximately 70% recurrence and 46% metastatic rates.

60 Cytogenetic Analysis of Myxoid Soft Tissue Tumors by Array-Based Comparative Genomic Hybridization

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Background: Myxofibrosarcoma is a major type of adult soft tissue sarcoma and has overlapping myxoid morphology with other myxoid soft tissue tumors such as myxoid liposarcoma. Array-based comparative genomic hybridization (aCGH) is a recently developed, feasible molecular technique that allows high throughput analysis of DNA copy numbers at high resolution throughout the whole genome. We investigated overall chromosomal aberrations of myxofibrosarcoma and other myxoid soft tissue tumors using aCGH to delineate genomic profiles of these entities.
Design: Genomic DNA was extracted from fresh-frozen tissues of 8 myxofibrosarcomas, 10 myxoid liposarcomas, 2 intramuscular myxomas, and one extraskeletal myxoid chondrosarcoma. The DNA was labeled with fluorochromes and hybridized onto an array consisting of 1440 bacterial artificial chromosome clones covering the entire human genome. Scanned array signals were analyzed, and test/reference fluorescence ratio of each sample was determined. Increased or decreased DNA copy number was considered to be over or below the thresholds set at log2ratio of 0.25 and -0.25, respectively.
Results: DNA copy number changes were found in all myxofibrosarcomas examined, but no alterations were found in seven of 10 myxoid liposarcomas or other myxoid tumors. In myxofibrosarcoma, most frequent DNA copy number changes were gains at 7p (4 cases) and 12q (4 cases), and a loss at 13q (4 cases). The number or pattern of the changes did not seem to correlate with histologic grades of the tumor. In 3 myxoid liposarcomas, only a few DNA copy number changes such as gains of chromosomes 8 (two cases) and 5 (one case) and a loss at 21q (one case) were detected.
Conclusions: Recurrent chromosomal imbalances frequently detected in myxofibrosarcoma but not in other myxoid tumors by our aCGH are in keeping with the view that myxofibrosarcoma has a distinct cytogenetic background from other myxoid soft tissue tumors.

61 Analysis of ALK Abnormalities in Inflammatory Myofibroblastic Tumors: An Immunohistochemical (IHC) and Fluorescence In Situ Hybridization (FISH) Study

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Background: IMTs are rare mesenchymal neoplasms that have an unpredictable clinical course. Moreover, they can be difficult to distinguish from a variety of other spindle cell neoplasms. Anaplastic lymphoma kinase (ALK) has been implicated in the pathogenesis of at least a subset of these tumors.
Design: Paraffin embedded, formalin-fixed tissue sections of 9 cases of well-characterized IMT were utilized for analysis of ALK expression by IHC and 2p23 (ALK gene locus) rearrangement by FISH. In addition, we assessed the presence of the above abnormalities in a series of spindle cell neoplasms that may mimic IMT including fibromatosis (n=4), nodular fasciitis (n=3), GIST (n=2), leiomyosarcoma (n=2), rhabdomyosarcoma (n=2), leiomyoma (n=1), fibrosarcoma (n=1), synovial sarcoma (n=1) and MFH (n=1).
Results: ALK positivity by IHC was observed in only 3/9 (33%) IMTs. Two of these three immunoreactive cases also showed evidence of 2p23 rearrangement by FISH, while the third immunoreactive case showed a deletion at the centromeric site of the breakapart 2p23 probe, possibly reflecting a cytogenetic abnormality subsequent to a translocation. The remianing IMTs as well as the other 17 spindle cell neoplasms examined were ALK negative by IHC and showed no 2p23 abnormalities by FISH.
Conclusions: In this small series of well-characterized IMTs, 33% of cases were immunoreactive to ALK, and all three immunoreactive cases were shown to harbor 2p23 abnormalities by FISH. None of the ALK-negative cases showed 2p23 rearrangement. ALK or 2p23 abnormalities were not detected in any of the other spindle cell neoplasms evaluated in this study.

62 Can Immunohistochemistry for Human Telomerase Reverse Transcriptase Catalytic Subunit (hTERT) Distinguish Benign from Malignant Soft Tissue and Bone Tumors?

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Background: Expression of telomerase, an enzyme that extends telomere specific DNA repeats, has been demonstrated in stem cells, carcinomas, and sarcomas. However, evaluation of telomerase activity in routinely fixed tissues by traditional assays is very difficult. Recently, paraffin-reactive antibodies to hTERT have become available and have been shown to accurately reflect telomerase activity. Only a small number of mesenchymal tumors have been evaluated for hTERT expression. We hypothesized that hTERT expression would be frequently present in sarcomas, but not in benign tumors

Design: Sections from 143 bone/soft tissue tumors were immunostained for hTERT (44F12, 1:20, Novacastra) using steam HIER and Dako Envision system. Tumor types are detailed in Table 1. Normal lymphocytes served as positive internal controls. Positive cases showed "speckled" nuclear/nucleolar staining in >10% of cells. Fisher's Exact Test was used.

Results: hTERT expression was seen in 9/71 (13%) benign and 33/72 (46%) malignant tumors (p= 0.00002). hTERT was positive in ≥50% of OS, MPNST, LPS, and AS. All positive sarcomas were high-grade. Among benign tumors, only schwannomas and chondromas were positive in >30% of cases. Positive normal tissues included lymphocytes and multinculeated histiocytes.

Telomerase IHC in Bone/Soft Tissue Tumors

Benign	Positive (%)	Malignant	Positive (%)
Hemangioma	1/8 (13)	Angiosarcoma	5/10 (50)
Lipoma	0/11 (0)	Liposarcoma	5/8 (63)
Reactive fibrosis	0/10 (0)	Fibromatosis/LG Fibrosarc	0/12 (0)
Schwannoma	5/13 (38)	MPNST	11/17 (65%)
Chondroma	3/9 (33)	Chondrosarcoma	2/11 (18)
Osteoid osteoma/osteoblastoma	0/20 (0)	Osteosarcoma	10/14 (71)
All benign	9/71 (13)	All malignant	33/72 (46)

Conclusions: hTERT expression is significantly more common in sarcomas as compared with benign tumors. Restriction of hTERT expression to high-grade lesions, and its absence in low-grade sarcomas (including the well-differentiated component of dedifferentiated liposarcoma), suggests that telomerase activiation is a late event in sarcoma progression. Importantly, however, hTERT can be expressed in some benign tumors, notably schwannoma and chondroma, and it is doubtful that hTERT expression alone will allow the discrimination of benign from malignant soft tissue/bone tumors. hTERT expression is often only focal and may require careful evaluation of an entire section, suggesting the distinct possibility of false negative staining with tissue microarray sections.

63 The Utility of Cytogenetics, FISH, and RT In Situ PCR for the Diagnosis of Synovial Sarcoma

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Background: The diagnosis of synovial sarcoma is typically based on H&E and immunohistochemical analyses. However, in cases, an unequivocal diagnosis cannot be made. In such circumstances, detection of the X-18 chromosomal translocation can aid in the diagnosis. The purpose of this study was to analyze the utility of cytogenetics, FISH, and RT in situ PCR for the X-18 chromosomal translocation characteristic of synovial sarcoma.

Design: We studied 12 cases of synovial sarcoma diagnosed by H&E and immunohistochemistry (IHC), 21 negative controls (a variety of benign and malignant soft tissue tumors), and 5 cases of possible synovial sarcoma based on H&E and immunohistochemistry.

Results: Cytogenetics testing was positive in 8/8 synovial sarcomas. FISH was positive in 3/8 synovial sarcomas and 0/8 of the negative controls. RT in situ PCR detected the fusion mRNA in 12/12 of the synovial sarcomas and 5/5 of the cases suspicious for this diagnosis based on IHC and H&E analysis; 0/21 negative controls were positive by RT in situ PCR.

Conclusions: We conclude that FISH is specific but not sensitive for the X-18 translocation of synovial sarcoma, and that the PCR-based method for cDNA detection of the translocation mRNA is highly sensitive and specific and allows direct correlation of the molecular findings with the histologic features of the lesion.

64 Myxoid/Round Cell Liposarcoma: Beyond the Round Cell Paradigm

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Background: Myxoid and round cell liposarcoma (LPS), once thought to be distinct entities, are now believed to represent, respectively, the low and high ends of a spectrum of tumor grade. Currently, the threshold of round cell content that is associated with more aggressive behavior is 5%. Assignment of grade based solely on the presence or absence of a given amount of round cell component fails to consider important aspects of non-round cell histology, including largely neglected high grade components, especially the pleomorphic spindle cell pattern described by Stout as poorly differentiated myxoid LPS, as well as morphologic overlap with pleomorphic LPS with myxoid stroma.

Design: Twenty-three cases of myxoid LPS were reviewed and compared with five cases of pleomorphic LPS with myxoid stroma. All cases were scored according to 16 morphologic indices, including nuclear grade, pleomorphism, mitoses, round cell component, and necrosis. Analysis of all cases by polymerase chain reaction (PCR) to amplify fusion sequences and determine molecular genetic similarities is currently underway.

Results: Of the 23 myxoid LPS, five had the Stout spindle cell pattern. They retained the defining characteristics of myxoid LPS (abundant myxoid stroma, plexiform vascular pattern, peripheral lobular condensation, mucin pools), and two of these had an appreciable round cell component. However, all five were dominated by a pleomorphic spindle cell morphology. Of the remainder, five were pure round cell LPS, six had both myxoid and round cell components, and seven were pure low grade myxoid LPS. An adverse clinical event (metastasis, recurrence, or death of disease) was identified in 14% of the pure myxoid, 45% of the myxoid/round cell, and 60% of the Stout spindle cell myxoid LPS (median follow-up 28 months, range 2-120 months). All five cases categorized as pleomorphic LPS had moderate to abundant myxoid stroma and morphologic overlap with myxoid LPS.

Conclusions: The morphology of myxoid LPS is broad and includes fairly pleomorphic tumors. It is likely that the degree of round cell differentiation is not the only parameter that must be considered when assigning a grade to myxoid LPS. We anticipate that the molecular data will show that some cases of pleomorphic LPS are perhaps variants of myxoid LPS.

65 Promoter Methylation Profiling of Tumor Suppressor Genes in Pleomorphic Sarcoma

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Background: Pleomorphic sarcoma, aka, malignant fibrohistiocytoma (MFH), is a high-grade mesenchymal malignancy, composed mainly of transformed fibrocytes and histiocytes. Most genetic changes frequently altered in epithelial malignancies, such as p53 and ras mutations, are rarely seen in sarcomas. To our knowledge, studies of epigenetic changes in soft tissue tumors are only handful. Our previous study revealed low methylation frequency for HIC-1 in pleomorphic sarcoma. To further understand the role of epigenetic alterations in sarcomatogenesis, we have expanded our study in assessing methylation profiles of 16 candidate tumor suppressor genes (TSG) in 20 cases of pleomorphic sarcomas.

Design: Genomic DNA was obtained from frozen tissue blocks of 20 cases of well-characterized high-grade pleomorphic sarcomas. DNA was chemically converted with bisulfite before amplication. Promoter methylation of *p14, p15, p16, p73, APC, CDH1, DAPK, SOCS-1, ER, RASSF1a, hMLH-1, RAR-beta, TIMP3, VHL, GSTP* and *MGMT* was detected using methylation-specific PCR.

Results: Methylation of at least one TSG was seen in all the cases of MFH. Approximately 85% (17/20) cases had three or more genes methylated. Methylation of *DAPK*(78%) and *SOCS-1* (72%) were most frequently seen and the association between methylation of *DAPK* and *SOCS-1* was statistically significant (p<0.05). Methylation of *p14, p15* and *p73* were 50%, 50% and 44% respectively. There was significant dissociation (mutually exclusive) between methylation of *p14* and *p73* (kappa = 0.90). Methylation of *p16, RASSF1a, RAR-beta, CDH1, HIC-1, MGMT* and *APC* was not frequent (6-33%) in MFH. No methylation of *TIMP3, VHL* and *GSTP* was detected. There was no significant association between methylation of any TSG with patient's sex, age, tumor size and location and clinical stage.

Conclusions: Our study demonstrates that epigenetic change through promoter methylation is a frequent event in pleomorphic sarcoma. Most frequently altered genes are *DAPK*, an apoptotic inhibitor, and *SOCS-1*, a JAT/STAT pathway suppressor. Although p53 mutation is a rare event in MFH, methylation of *p14 or p73*, two important elements involved in p53-pathway, is frequent but mutually exclusive in majority of MFH. Our study indicates that epigenetic alterations of JAK/STAT, p53, and apoptotic pathways may play important roles in development of high grade pleomorphic sarcoma.

66 Array-CGH Analysis of Chondromyxoid Fibroma

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Background: Chondromyxoid fibroma (CMF) is a rare benign cartilaginous tumor of bone, mainly occurring in the second decades and affecting long bones. Karyotype studies showed both balanced and imbalanced recurrent rearrangements involving 6p23-25, 6q12-15 or 6q23-27 to be associated with CMF. We have used array comparative genomic hybridization (array-CGH) in order to detect genomic imbalances that are specific for CMF.

Design: DNA was isolated from 6 frozen tumor samples of CMF containing >70% tumor cells as controlled by frozen section. DNA-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 recurrent alterations were found for CMF, though small amplifications and deletions (1-3 adjacent clones) were present in individual cases. None of these except for one matched with translocation sites of previously published cases. One case however showed multiple interstitial deletions on chromosome 3q and 6q.

Conclusions: No recurrent genomic imbalances (gains/losses) were found indicating that in-line with the literature data a balanced rearrangement in stead of substanial genomic imbalances seems to be the most likely genomic alteration leading to CMF. This is of note given the pleomorphic nature of a subset of tumor cells.

67 A Differentially Expressed Gene-Set Differentiates Chondromyxoid Fibroma from High-Grade Central Chondrosarcoma

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Background: Chondromyxoid fibroma (CMF) is a rare cartilaginous bone tumor composed of lobules of chondromyxoid matrix surrounded by cellular fibrous areas. The characteristically presence of polygonal - atypical cells creates difficulty in the differential diagnosis with high-grade central chondrosarcoma (HGCCS) especially in biopsy specimens. No markers of use for differential diagnosis have ever been reported.

Design: RNA isolated from 7 CMF and 12 HGCCS was hybridized on a 9k- cDNA microarray enriched for cartilage specific genes and printed in duplicate. For data analysis *Linear Model for Microarray Analysis* was used. Immunohistochemistry allowed for verification of differentially expressed genes on a larger series of CMF (n=20) and HGCCS (n=39). Antibodies directed against CD166, p16 and Cyclin D1 were applied in a two-step protocol.

Results: Fifty-six genes showed to be differentially expressed (p<0.01 after false discovery rate correction). CD166 and Cyclin D1 were higher expressed in CMF versus HGCCS, which was immunohistochemically verified (p<0.05). p16, known to be low expressed in high grade chondrosarcomas was also evaluated, because of its inhibitory effect on Cyclin D1. Its expression was significantly higher in CMF (p<0.01).

Conclusions: We have identified and validated 3 possible markers for the differential diagnosis of CMF versus HGCCS, which can be used on a routine basis: Cyclin D1, CD166 and p16. Higher expression of Cyclin D1 is present in CMF, which is in general unusual for a benign tumor. However its co-occurrence with high p16 expression might balance their antagonistic actions. The crucial role for loss of p16 expression in HGCCS is confirmed.

68 The Wnt/ß-Catenin Signaling Pathway in Synovial Sarcoma: Mutational and Functional Analysis

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Background: Nuclear ß-catenin staining (consistent with Wnt/ß-catenin pathway activation) is observed in 30% to 60% of synovial sarcoma (SS), primarily in monophasic cases or in the spindle cell component of biphasic cases. The epithelial component of biphasic cases shows cytoplasmic and membranous expression. Activating mutations in this pathway have been sporadically reported in SS, including in the APC (8%) and ß-catenin (8%) genes. To better understand the role of this signaling pathway in SS, we are performing a systematic mutational screen in 40 SS with cDNA microarray data along with functional studies in SS cell lines.

Design: The ß-catenin, APC, Axin1, and Axin2 genes are being screened for mutations by direct sequencing in 40 SS tumors and 3 SS cell lines (SYO-1, HS-SY-II, FUJI). To evaluate the cellular effect of Wnt/ß-catenin signaling in SS, activating (S33Y ß-catenin) or inhibitory (dominant negative LEF-1) constructs were transfected into SS cell lines, and their effects on phenotype and on activation of a co-transfected E-cadherin promoter plasmids were studied. E-cadherin has been reported as a target of repression by Wnt/ß-catenin signaling in other cell types.

Results: Seven mutations (2 each in ß-catenin, Axin1, and Axin2, and 1 in APC) have been identified in 6 tumors (one case had mutations in both ß-catenin and Axin1) and SYO-1 cells were found to contain a ß-catenin mutation. Blocking the Wnt/ß-catenin signaling pathway in SYO-1 cells results in activation of the E-cadherin promoter, and conversely, activation of the pathway in HS-SY-II cells reduced E-cadherin promoter activity. At the morphological level, SYO-1 cells stably transfected with dominant negative LEF-1 showed a drastic change from spindle shape to epithelial and polygonal shape with reduced proliferation.

Conclusions: In addition to the previously reported mutations in ß-catenin and APC, we identify for the first time Axin1 and Axin2 mutations in SS. Activation of Wnt/ß-catenin signaling may influence tumor phenotype both in terms of tumor proliferation and cellular morphology. Supervised clustering of 40 SS to define the expression profile of Wnt/ß-catenin activation in this sarcoma (based on pathway mutation status and/or nuclear ß-catenin accumulation) is in progress and should help to identify the key Wnt/β-catenin target genes that regulate these phenotypic differences.

69 CDK4 Amplification in Central Chondrosarcoma Is Associated with Tumor Progression

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Background: Central chondrosarcomas are malignant cartilaginous tumors centered in the medullar cavity. Previously, p16, a cyclin-dependent kinase inhibitor that binds CDK4, was demonstrated to be lost in central chondrosarcoma upon tumor progression. In addition, using array-Comparative Genomic Hybridization (aCGH), gain of the 12q13 chromosomal region containing CDK4 was found in 4 out of 12 central chondrosarcomas. This was associated with higher expression levels of CDK4 as shown by cDNA array analysis. During G1 phase, CDK4 forms a complex with cyclin D, which promotes progression to the S phase. The proto-oncogene c-MYC encodes a transcription factor implicated in the regulation of cellular proliferation, differentiation, and apoptosis. The promoter of the CDK4 gene contains four highly conserved MYC binding sites. In this study we investigated the role of c-MYC and CDK4 in central chondrosarcoma.

Design: mRNA expression levels of CDK4 and c-MYC were studied using quantitative RT-PCR in phalangeal enchondromas (n=7) and central chondrosarcomas (grade 1 n=11, grade II n=7 and grade III n=9). Two samples of normal cartilage and 4 growth plate samples were used for comparison. Tumor samples with 12q13 region gain, ascertained by array-CGH, were compared with tumors without gain. Interphase fluorescent *in situ* hybridization was performed with a probe for the 12q13 region (RP11-571M6), containing the CDK4 gene, and a centromere-12 specific probe, as well as for c-MYC on 8q24 (RP1-80k22) and a centomere-8 specific probe.

Results: Tumors showed upregulation of CDK4 compared to growth plate samples (p=0,001). mRNA levels increased with increasing histological grade (p=0,003). Tumors with amplification of 12q13 by CGH (n=4) showed higher expression levels than tumors without amplification (n=8) (p=0,001). In contrast, no differences in c-MYC expression levels were found.

Conclusions: We confirmed that central chondrosarcomas harboring amplifications on 12q13 express higher levels of CDK4. Expression of the oncogene c-MYC does not seem to be important in chondrosarcoma. In addition to loss of p16, increased CDK4 expression is associated with increasing histological grade, underlining the crucial role of these cell-cycle regulating molecules in chondrosarcoma progression.

70 GLUT-1 Expression and Enhanced Glucose Metabolism Are Associated with Tumor Grade in Bone and Soft Tissue Sarcomas: A Prospective Evaluation by [F-18]-Fluorodeoxyglucose Positoron Emission Tomography

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Background: Positron emission tomography (PET) can be used to measure tumor metabolism in sarcomas by measuring the standard uptake value (SUV) of (F-18) fluorodeoxyglucose (FDG) and enhanced uptake reflects tumor aggressiveness and has been used for diagnosis and grading of tumor type. The glucose transfer mediated by glucose transporter protein 1 (GLUT-1) plays a pivotal role in the development and malignant behavior of cancer cells and is overexpressed in cancer cells and to promote glucose metabolism and FDG accumulation. However, GLUT-1 overexpression has never been clearly identified in bone and soft tissue sarcomas (B&SFS), and the relationship between GLUT-1 overexpression and FDG uptake or pathologic background has never been systematically analyzed. The significance of GLUT-1 overexpression in B&SFS in vivo, however has remained unexplored. The aim of the present study was to identify the histological variables, including tumor grade, cell proliferation, cell-cycle control integrity, and overexpression of GLUT-1, affect FDG PET values in patients with B&STS.

Design: We compared FDG-PET SUV in sixty cases of B&STS with GULT-1 expression as well as histopathological features, including tumor grade determined by tumor differentiation, tumor necrosis, mitosis, MIB-1 grade, and p53 expression. All patients had undergone PET before biopsy. Deparaffinized, formalin-fixed sections were immunostained with antibodies to Glut-1 (A3536), MIB-1, and p53. Grade for intensity of GLUT-1 staining was given a core of 0 (0%), 1 (1-9%), 2 (10-29%), or 3 (30%<).

Results: GLUT-1 was expressed in 92.0% of all tumors. GLUT-1 intensity correlated positively with FDG-PET SUV (P<0.0001) and was associated with MIB-1 grade (P<0.0001), mitotic grade (P<0.0001), and differentiation (P<0.0001). High-grade tumors had significantly higher FDG-PET SUV than low-grade tumors (P<0.05).

Conclusions: B&STS have an enhanced glucose metabolism which is correlated with tumor grade. The enhanced glucose metabolism is also associated with an increased GLUT-1 staining intensity as well as the p53 overexpression by the tumor, suggesting a role for this enhanced metabolic response in the evaluation prior to therapy.

71 Serine/Threonine Kinase Mirk/Dyrk1B Mediates Survival in Rhabdomyosarcomas

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Background: The serine/threonine kinase Mirk/Dyrk1B is a critical regulator of myoblast growth control, differentiation and cell survival and is highly expressed in normal skeletal muscle. Mirk is also expressed at high levels in many solid tumors, where it facilitates cell survival. The purpose of this study was to assess the potential of Mirk as a therapeutic target in rhabdomyosarcoma (RMS) by determining if Mirk expression, kinase activity and survival functions are maintained in RMS tumors.

Design: Immunohistochemistry was used to assess the expression of Mirk protein in paraffin embedded sections from 16 cases of RMS (8 alveolar, 4 embryonal, 4 pleomorphic) and in multiple cell lines. Western blotting was used to analyze the induction of Mirk under mitogenic stress conditions and immune complex kinase assays were used to determine the relative kinase activity of Mirk immunoprecipitated from multiple RMS cell lines. RNA interference, in conjunction with colony formation, TUNEL and annexin binding assays, was used to assess the role of Mirk in rhabdomyosarcoma cell survival.

Results: Mirk was expressed in each case of rhabdomyosarcoma examined as well as in multiple cell lines derived from both embryonal and alveolar RMS. Mirk was predominately located in the cytoplasm of RMS tumors, which is consistent with its cell survival function. Mirk was induced and activated under mitogenic stress conditions in RMS cells in culture, where it had enhanced function as a kinase compared to Mirk isolated from C2C12 myoblasts. Knockdown of Mirk by RNA interference reduced the viability of RD embryonal rhabdomyosarcoma cells and RH30 alveolar rhabdomyosarcoma cells 3 to 4 fold in multiple colony formation experiments. Depletion of Mirk in the context of serum deprivation significantly increased cell death by apoptosis as shown by increased numbers of TUNEL positive cells and by increased binding of annexin V (X^2-square, $p < 0.0001$).

Conclusions: Mirk kinase is a critical mediator of cell survival in rhabdomyosarcoma. The survival function of Mirk and its wide expression in rhabdomyosarcoma suggest that Mirk may be a novel therapeutic target in RMS.

72 Frequency of *USP6* Locus Rearrangements in Myositis Ossificans, Brown Tumors and Cherubism

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Background: *USP6* rearrangements with several partner genes have been recently identified in primary but not in secondary aneurysmal bone cysts (ABC). Several lesions show morphologic features that overlap with ABC, including myositis ossificans, brown tumor and cherubism. The aim of this study was to assess whether these lesions also harbor *USP6* rearrangements.

Design: Twelve cases of myositis ossificans, six of brown tumor, and three of cherubism from the Mayo Clinic files were studied for the presence of *USP6* rearrangements on paraffin-embedded tissues by fluorescence in situ hybridization (FISH) using a probe flanking the *USP6* locus on chromosome 17p13.

Results: *USP6* rearrangements were identified in two cases of myositis ossificans with classic clinico-radiologic features. No case of brown tumor or cherubism showed *USP6* rearrangements.

Conclusions: We demonstrate that a subset of myositis ossificans with classic clinicoradiologic features contain clonal *USP6* rearrangements and likely represent variants of soft tissue ABC. In contrast, no *USP6* rearrangements were found in cherubism and brown tumors, supporting the prevailing view that these lesions are distinct biologic entities.

73 The Distinct Morphology and Structure of Tissue Engineered Bone and Cartilage Derived from Mesenchymal Stem Cells

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Background: The production of engineered bone and cartilage from mesenchymal stem cells is a rapidly developing field. Potential applications include the treatment of degenerative joint diseases, as well as the treatment of traumatic and surgical bone injury. As engineered tissue is implanted, pathologists will play a role in evaluating the integration, biocompatibility, ultrastructure and performance of the engineered tissue, especially in explanted specimens. Our purpose is to introduce pathologists to the distinctive morphology of engineered bone and cartilage tissue such that it is recognized aptly in clinical practice. There have been extensive publications addressing

tissue engineering over the last decade, however very little has been directed at the practicing pathologist.
Design: Human mesenchymal stem cells were grown in vitro in pellet culture (for chondrocyte differentiation) and monolayer (for osteogenic differentiation) for three weeks in chondrogenic and osteogenic media conditions. The cultures were then harvested, paraffin-embedded, and routine histology with hematoxalin and eosin staining was performed. Additional stains including saffranin-O for glycosaminoglycans and Von Kossa for calcium phosphate were performed on the engineered cartilage and bone, respectively, to highlight matrix production as a surrogate marker for differentiation. The morphology of the engineered bone was compared to that of native fetal and adult cartilage.
Results: The engineered tissue demonstrates similar morphology to native cartilage and bone by routine hematoxalin and eosin staining. Of note, the cell to matrix ratio of the engineered tissue lies between that of fetal and adult cartilage or bone. Other parameters such as mineralization activity and the formation of extracellular matrix, as demonstrated by Von Kossa and saffranin-O staining, paralleled that of native tissue.
Conclusions: In the near future human tissue engineered explants will require pathologic evaluation. The engineered cartilage and bone have a characteristic cell to matrix ratio, which lies in between that of fetal and adult native tissues. The phenotypic differences are highlighted here with the purpose of introducing surgical pathologists to the histology of a biomaterial that they may soon encounter in practice.

74 Fluorescence In-Situ Hybridization (FISH) Assay for Ewing's Sarcoma/PNET on Formalin-Fixed Paraffin-Embedded (FFPE) Tissue: A Pilot Study of Genetically Confirmed Cases

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Background: The diagnosis of malignant small round cell tumors including Ewing's sarcoma(ES)/PNET requires the aid of ancillary studies including immunohistochemical stains and, more recently, molecular confirmation. A FISH-based assay to detect diagnostic translocations has recently become available (LSI ® EWSR1 breakapart rearrangement probe) and may be applied to routinely processed FFPE tissues. We conducted this pilot study to determine the potential utility of using this FISH probe in the diagnosis of ES/PNET.
Design: Three cases of histologically, immunohistochemically and genetically confirmed ES/PNET were retrieved from the Hartford Hospital surgical pathology files from 2000-2001. Additional sections were prepared from representative paraffin blocks and were subjected to FISH analysis using the LSI ® EWSR1 probe (Vysis ®). Since this breakapart rearrangement probe hybridizes with EWSR1 (22q12) it will detect either the t(11;22) or t(21;22) translocation. Two additional cases of ES/PNET without genetic confirmation and four cases of non-ES/PNET round cell sarcomas were also probed.
Results:

	Diagnosis	IHC	PCR	FISH
1	ES/PNET	cd99,nse,vim,ck$^+$	EWS/FLI-1	positive(<10 cells present)
2	ES/PNET	cd99,nse,vim$^+$	EWS/FLI-1	positive(97% of cells)
3	ES/PNET	cd99,nse$^+$	EWS/FLI-1	positive(99% of cells)
4	ES/PNET	cd99,nse,vim$^+$	nd	positive(96% of cells)
5	ES/PNET	cd99,nse,vim$^+$	nd	positive(98% of cells)
6	PDSS[a]	cd99,nse,vim$^+$	nd	negative
7	RCS-NOS[b]	cd99,fli-1 (-)	nd	negative
8	RCS-NOS[b]	cd99,fli-1 (-)	negative	negative
9	RCL[c]	nd	nd	negative

nd=not done; a=poorly differentiated synovial sarcoma; b=round cell sarcoma, not otherwise specified; d=round cell liposarcoma.
Conclusions: Since all three genetically confirmed ES/PNET were FISH positive (as well as both of the histologically-suspect and IHC-confirmed ES/PNET), and the four cases of non-ES/PNET were FISH negative, this new LSI ® EWSR1 probe may prove potentially useful in the differential diagnosis of small round cell tumors, especially when fresh tissue is not available.

75 Cluster Analysis of Immunohistochemical Profiles in Melanoma and MPNST: Phenotypic Continuum and Diagnostic Strategy

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Background: Morphologic and immunophenotypic overlap between melanoma and MPNST can present a diagnostic challenge, particularly in tumors that are negative for HMB45 and Melan A. To test the usefulness of immunohistochemistry in the differential diagnosis, we studied a series of melanomas of varying subtypes and MPNST with a panel of melanocytic and neural markers by cluster analysis.
Design: Tissue microarrays containing 42 epithelioid melanomas (EM), 30 desmoplastic or spindled melanomas (DSM), and 26 MPNST were immunostained with nine selected antibodies. Intensity of staining was scored as negative, weak, or strong with attention to diffuseness and cellular localization. Tabulated data were analyzed with cluster analysis software which divided the tumors into statistically similar groups based on any positivity, strong or weak.
Results: Cluster analysis primarily divided the tumors into two large groups based upon HMB45 and/or Melan A reactivity. The positive group (n = 37) consisted entirely of melanomas, the majority (81%) being EM. The other group consisted almost entirely of HMB45 and Melan A negative tumors and clustered into three subgroups with the following predominant phenotypes: **1)** S100+, Nestin+, NGFR+, Clusterin- (13 DSM, 5 EM, 3 MPNST); **2)** Clusterin +, and mostly negative for S100, Nestin, and NGFR (11 MPNST, 2 EM, 0 DSM); and **3)** S100+, Nestin+, NGFR+, Clusterin+ (12 MPNST, 7 DSM, 1 EM). PGP9.5, Fascin, and Collagen IV were evenly distributed among the tumors and therefore had little effect on clustering.
Conclusions: These data reflect a continuum of immunophenotypic differentiation consisting of loss of melanocytic and gain of neural differentiation between EM at one end of the spectrum and MPNST at the other. EM is usually HMB45+, Melan A+, NGFR-, and Clusterin-, while DSM is usually HMB45-, Melan A-, NGFR+, and Clusterin-. MPNST shows two major immunophenotypes: one well-differentiated (S100+, Nestin+, NGFR+), the other less differentiated (S100-, Nestin-, NGFR-). However, unlike melanoma, the majority of both types of MPNST are Clusterin+. Although immunophenotypic overlap exists, a panel consisting of S100, Nestin, NGFR, and Clusterin appears to have diagnostic utility in distinguishing between HMB45/Melan A-negative melanoma and MPNST.

	Percentages of Strongly Positive Tumors					
	HMB45	Melan A	S100	Nestin	NGFR	Clusterin
EM	60	67	95	71	19	24
DSM	10	27	100	73	77	23
MPNST	0	0	54	35	35	85

76 Neurofibromatosis Type 1-Related Gastrointestinal Stromal Tumor: Special Emphasis on KIT and PDGFRA Mutations, Loss of 14q and 22q, and Activation of MAPK Pathway

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Background: Multiple GISTs rarely occur in the patients with neurofibromatosis type 1 (NF-1). The mutational inactivation of NF1 gene, which is present in NF-1 patients, results in the hyperactivation of Ras, leading the activation of downstream anti-apoptotic or growth signaling such as mitogen activated protein kinase (MAPK). Most of sporadic GISTs have a gain-of-functional mutation of KIT or platelet-derived growth factor receptor alpha (PDGFRA) with the activation of downstream RAS-Raf-MAPK pathway. Allelic losses of 14q and 22q are also common features in sporadic GISTs. However, molecular pathogenetic mechanisms of NF-1 related GISTs (NF-1 GISTs) remains unclear.
Design: In this study, we analyzed the c-kit and PDGFRA mutation, activation (phosphorylation) of MAPK p44/42, loss of heterozygosity (LOH) at 14q and 22q in NF-1 GIST. Thirty-one GISTs from five NF-1 patients and 10 sporadic tumors (10 patients) were examined.
Results: Most of NF-1 GISTs occured in small intestine. Neither KIT nor PDGFRA mutation was detected in 25 NF-1 GISTs. In contrast, KIT exon 11 mutations were detected in 7/10 (70%) sporadic GISTs. Immunohistochemical expression of phospho-MAPK p44/42 was more frequently found in NF-1 GISTs (21/25 cases; 84%) as compared to sporadic GISTs (6/10; 60%). Among the informative cases, LOH at 14q and 22q was seen in 7/8 (87.5%) and 5/12 (41.7%) of NF-1 GISTs, respectively. LOH at 14q and 22q was present in 4/8 (50.0%) and 7/8 (87.5%) of sporadic GISTs, respectively. These losses were observed in various sized of NF-1 GISTs, including small tumors less than 1 cm in size.
Conclusions: Our results suggest that LOH at 14q and 22q may contribute to the relatively early event in the tumorigenesis of NF-1 GIST as well as sporadic GIST. KIT and PDGFRA mutations are very rare event in NF-1 GIST. Rather, activation of Ras-MAPK pathway, which might be related to inactivation of NF1 gene, may play an important role in tumorigenesis of NF-1 GIST.

Breast

77 Chromosomal Alterations in Hyperplastic Regions in the Breast Depend upon the Presence of Coexisting Cancer

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Background: The role of ductal hyperplasia, usual (UDH) and atypical (ADH), as precursors to breast carcinoma (Ca) is still unclear. Therefore, we studied genomic abnormalities by fluorescence in situ hybridization (FISH) in 27 cases of hyperplasia (6 ADH) with coexisting Ca, and 17 (4 ADH) who never had Ca.
Design: Discrete areas of normal epithelium, UDH, ADH, and Ca were marked on sections for FISH. These were hybridized with 12 FISH probes in 4 multi-color panels, for 1p12, centromere (cen) 8, cen 11, and cen 17 (Breast Aneusomy Probe Set, Vysis/Abbott), TOP2A (17q21.2), MYC (8q24.21), MYH11 (16p13.11), CDH1 (16q22.1), cen 16, 1p36.3, 1q25, and COX2 (1q31.1). 20-40 cells were enumerated for each probe for each marked region, and percentage of cells with gain (>2 signals) or loss (<2 signals) of each locus determined. Student's t-test was used to compare the percentages of cells with copy number changes of each locus between histological categories.
Results: As expected, significant gains of nearly all loci were found in Ca versus normal ($p<.05$ for all loci except TOP2A). UDH or ADH versus normal regions showed significant gains for 1p12 ($p=.02$), cen 8 ($p<.001$), cen 11 ($p<.001$), cen 17 ($p<.001$), TOP2A ($p<.001$), MYC ($p<.001$), & MYH11 ($p=.007$). Normal regions showed no significant changes, even in the presence of coexisting Ca, but UDH and ADH showed gain of 1p12 ($p=.02$), 1q25 ($p=.05$), and COX2 ($p=.002$), and loss of cen 11 ($p<.001$), cen 17 ($p=.008$), TOP2A ($p=.004$), and MYC ($p=.02$) in the presence of coexisting Ca **compared to UDH and ADH without Ca**. The effect was greater if the coexisting Ca was located on the same section as UDH or ADH. ROC analysis for the presence of coexisting Ca by gene copy changes in UDH or ADH predicted that greater than 80% sensitivity and specificity might be achieved with several probe combinations (e.g. 1p12 and CEP 11), and greater than 85% sensitivity and specificity with the combination of 1p12, cen 11, and MYC.
Conclusions: Hyperplastic lesions in the presence of coexisting Ca are molecularly distinct from hyperplastic lesions without Ca. This finding has several implications with regard to carcinogenesis, and suggests that occult Ca might be detected by FISH analysis of hyperplastic lesions in breast biopsies. More studies on larger cohorts will be needed to verify these findings.