Missing observations in asplenia and polysplenia cases

Absent observation	Asplenia n(%)	Polysplenia n(%)	Total n(%)
Spleen position	-	21(36)	21(36)
Spleen number	-	46(79)	46(79)
Lung lobation	9(24)	13(22)	22(23)
Liver morphology	13(35)	28(48)	41(43)
Stomach position	20(54)	38(66)	58(61)
Intestinal rotation	9(24)	8(14)	17(18)
Cardiac position	24(65)	46(79)	70(74)
Atrial isomerism	26(70)	46(79)	72(76)
Systemic venous return	21(57)	21(36)	42(44)
Pulmonary venous return	26(70)	51(88)	77(81)

No statistically significant difference was found in maximum coronary artery plaque thickness or per cent occlusion of coronary arteries. Plaque morphology, including extent of calcification, number of inflammatory cells, and lipid content, was similar in the two groups.

Conclusions: Heart weight tended to be greater in OSA than in non-OSA patients but the difference did not reach statistical significance. However, among those patients with heart weight exceeding the upper 95% confidence limit of predicted values, excess heart weight was significantly greater in OSA patients compared to patients without OSA. We found no significant difference in ventricular wall thickness, coronary plaque burden or plaque composition between the two groups.

18 Epidemiology of Endocarditis: A 22-Year Autopsy Review

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Background: Infective endocarditis (IE) affects 10,000 to 20,000 persons in the United States every year. Epidemiologic factors include community and hospital bacterial flora and patient risk factors such as damaged native valves, prosthetic valves, intravenous drug abuse, and intracardiac catheters and devices. As the incidence of nosocomial bacteremia has increased, we hypothesized a correlative change in the epidemiology of IE at autopsy.

Design: Following IRB approval, a review of 6386 autopsy reports from 01/01/1990 to 09/30/2012 identified 69 cases of IE. We recorded patient demographics, clinical history, and culture results; supplemented missing data using original medical records; and calculated trends with linear regression models and hypothesis testing.

Results: The incidence of IE showed a modest but statistically significant increase over time ($P=0.03$; $r^2=0.16$). Median age was 54; males were more affected than females (2.5:1); blacks and whites were equally affected. Endocarditis was more likely to be left-sided only (54:8). Seven patients had left- and right-sided involvement, and 18 patients had multiple valves involved. Despite hospitalization, 21 cases (30.4%) were not diagnosed until autopsy. Nearly half of patients had a recent invasive procedure (46.4%), including dialysis (11) and cardiac surgery (6). Risk factors included known infection (34.8%), prosthetic valves (21.7%), damaged or repaired native valves (18.8%), impaired immunity (17.4%), prior endocarditis (14.5%), and intravenous drug abuse (8.7%). Common presentations were fever (44.9%), neurologic symptoms (39.1%), dyspnea (37.7%), and pain (31.9%). Of cultured organisms, *Staphylococcus aureus* (SA) was most frequent (51.6%), with 21 cases being methicillin resistant (MRSA). The incidence of MRSA increased over time ($P<0.01$; $r^2=0.30$). Other causative organisms were Enterococcus (15.6%); 2 of 10 were vancomycin resistant (VRE). There were equal numbers of coagulase negative Staphylococcus (CNS) and gram negative bacilli (6 each). Dialysis rates increased over time ($P<0.01$; $r^2=0.23$). Dialysis patient cultures grew SA (17 with 9 MRSA), Enterococcus (5 with 1 VRE) and CNS (1). SA was the causative organism at a higher rate in dialysis patients than in non-dialysis patients ($P<0.01$). Black patients were more likely to be dialysis-dependent ($P=0.02$) and have SA endocarditis ($P=0.02$).

Conclusions: Our study showed increasing incidences of endocarditis, MRSA endocarditis, and endocarditis in chronic dialysis patients. Specifically, SA was most likely to be the causative organism in black patients as well as in dialysis-dependent patients.

Bone & Soft Tissue

19 IDH1 and IDH2 Mutations in Chondrosarcoma – A Sequenom Mass Spectrometry Analysis of 53 Cases

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Background: Heterozygous mutations of isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) have recently been identified in cartilaginous neoplasms, including conventional central and periosteal cartilage neoplasms. These mutations occur at a single amino acid residue at R132 for IDH1 and R172 and R140 for IDH2. Mutations in these genes lead to impaired ability of IDH1 and IDH2 to catalyze the conversion of isocitrate to alpha ketoglutarate. This results in neomorphic enzymatic activity leading to production of the oncometabolite 2-hydroxyglutarate (2HG). In this study, we analyzed chondrosarcoma for IDH1, IDH2 and other mutations using high-throughput Sequenom-based analysis.

Design: Chondrosarcomas were genotyped for IDH1 and IDH2 mutations on the Sequenom Mass Array Platform. In addition, 271 recurrent point mutations across 27 genes were tested as part of the high throughput Sequenom Mass Array Platform panel.

Results: Fifty three chondrosarcomas were selected for the study. There were 30 females and 23 males. The age range was 18 to 77 years with a median of 55 years. Histologically, twenty one (21) were classified as grade I/III, 25 as grade II-III/III and 7 as Dedifferentiated chondrosarcoma. Twenty-six of 53 (50%) patients had mutations in IDH1 or IDH2. No other mutations were detected in the rest of the gene panel (*AKT1, AKT2, AKT3, ALK, BRAF, CDK4, CTNNB1, EGFR, ERBB2, FGFR2, FGR3, FLT3, GNAQ, HRAS, JAK2, KIT, KRAS, MAP2K1, MET, NOTCH1, NRAS, PDGFRA, PIK3CA, PIK3R1, PTPN11, RET, SMO*) by Sequenom Mass Array spectrometry.

Conclusions: IDH1 and IDH2 mutations appear to be genetic signatures in half of chondrosarcomas. Downstream effects of these mutations could unravel pathways which could lead to viable therapeutic options. Most common genetic mutations involving genes of the signal transduction pathways do not seem to play a role in the biology of chondrosarcoma.

20 TFE3 Gene Rearrangements Define a Distinct Subtype of Epithelioid Hemangioendothelioma (EHE)

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Background: Classic EHEs are characterized by epithelioid cells arranged in cords and single cells in a myxo-chondroid or sclerotic stroma, typically lacking well-formed vasoformative properties. At the genetic level they show recurrent t(1;3) translocation, resulting in *WWTR1-CAMTA1* fusion gene, in the majority of cases, which can be used to exclude other epithelioid vascular tumors. In the course of *WWTR1-CAMTA1* screening, we have encountered a fusion-negative subgroup that show distinctive morphologic features which were investigated for recurrent novel genetic abnormalities.

Design: *WWTR1-CAMTA1* fusion-negative epithelioid vascular tumors, the morphology of which did not fit with either classic EHE or epithelioid hemangioma were selected for this study. Based on an index case showing strong TFE3 immunoreactivity, FISH analysis for TFE3 gene rearrangement was applied to the index case as well as to 4 additional cases. A control group, including 17 epithelioid hemangiomas, 9 pseudomyogenic (epithelioid sarcoma-like) HE and 3 high grade epithelioid angiosarcoma were also tested.

Results: TFE3 gene rearrangement was identified in 5 patients (3 males, 2 females, mean age 35 years), located in soft tissue (trunk, 2, head and neck, 1, extremity 1) and bone (vertebra, 1), but in none of the controls. The tumors were composed of epithelioid endothelial cells with abundant cytoplasm, ranging from pale-vacuolated to glassy-eosinophilic, with mild to moderate nuclear pleomorphism, but which lacked the usually greater anaplasia typically seen in angiosarcoma. They consistently showed well-formed vascular channels, in addition to a variably solid, nested or focally cord-like architecture. All tumors expressed endothelial markers (CD31 and/or ERG), as well as strong nuclear TFE3, but were negative for cytokeratin and HMB45.

Conclusions: We are reporting a novel subset of EHE of soft tissue and bone occurring in young adults which show TFE3 gene rearrangement associated with strong immunoreactivity for TFE3. The morphologic hallmark for these tumors is the voluminous eosinophilic cytoplasm with mild to moderate cytologic atypia and distinct vasoformative features.

21 Endobronchial Lipoma – Clinicopathologic Analysis of 12 Cases Showing Benign Behavior and Lack of 12q13-15 Amplification, Despite Worrisome Morphological Features

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Background: While lipomatous neoplasms are common in soft tissue locations, they rarely involve the bronchial tree. In our experience, lipomatous tumors of the bronchial tree often contain enlarged, atypical-appearing stromal cells, raising the possibility of well-differentiated liposarcoma. The clinicopathological and molecular cytogenetic features of endobronchial lipomatous tumors have not been previously studied in detail.

Design: Cases were obtained by searching the consultation archives of the authors (ALF and TVC), and the surgical pathology archives of Mayo Clinic Rochester for cases coded as "lipoma", "lipomatous hamartoma" and "liposarcoma" involving the bronchus or lung. All cases were reviewed by two soft tissue pathologists (ALF and KJF). Clinical information was obtained from clinical records and referring clinicians, where applicable. Selected cases were studied with FISH for *CPM, HMGA1* and *HMGA2*.

Results: The tumors occurred predominately in men (91%), and patient age ranged from 44-80 years (mean 65 years). Most patients (80%) had a former or current history of heavy smoking (20-100 pack-years). Three patients had concurrent pulmonary squamous cell carcinoma, and one had a previous history of multiple lung cancers. Most lesions were discovered incidentally. The endobronchial lipomatous neoplasms showed areas of fibrosis containing scattered hyperchromatic stromal cells, suggestive of well-differentiated liposarcoma. However, all such cases failed to show amplification of *CPM/MDM2*, typical of well-differentiated liposarcoma, and all patients were either alive without evidence of disease or had died of other causes at last follow-up. Additionally, of seven cases tested for *HMGA1* and *HMGA2* rearrangement, four had rearrangement of *HMGA2* and one had rearrangement of *HMGA1*, consistent with the expected profile observed for ordinary lipomas.

Conclusions: Although the morphological features of endobronchial lipomatous neoplasms may suggest the possibility of well-differentiated liposarcoma, our clinical and molecular cytogenetic findings strongly suggest that these represent lipomas instead. Given the clinical importance of distinguishing benign from malignant adipocytic lesions in these anatomical locations, we recommend liberal use of ancillary molecular cytogenetic tests for the evaluation of problematic cases.

22 *Rb1* and *CDKNA2* Gene Deletions Do Not Predict Resistance to Neoadjuvant Chemotherapy in Patients with Osteosarcoma

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Background: Pathologic response to neoadjuvant chemotherapy predicts survival in osteosarcoma (OS) patients. However, as pathologic response can only be assessed after treatment is completed. Thus, a need exists for molecular predictors of chemotherapy response that might be used to guide therapy. Consequently, we have previously shown that immunohistochemical expression of P16 (the product of the *CDKNA2* gene) predicts response to chemotherapy in patients with osteosarcoma (OS) undergoing neoadjuvant therapy. The objective of the current study was to determine if copy number variations (CNV) of the *CDKNA2* and *Rb1* genes correlate with immunohistochemical expression of P16 and to determine whether CNV predict response or resistance to neoadjuvant chemotherapy.

Design: Pre-treatment genomic DNA was available from formalin fixed, paraffin-embedded tissue sections in 31 pre-treatment cases of OS. CNV was determined by array comparative genomic hybridization (CGH). Good pathologic response to neoadjuvant chemotherapy was defined as > 90% tumor necrosis in the post-treatment surgical specimen. Clinical, pathologic, and copy number changes of the *CDKNA2* and *Rb1* genes were correlated for their association with good or poor response to chemotherapy.

Results: Array CGH was informative in 23 of 31 OS tumors. Of 12 tumors which were P16 positive by IHC, 100% demonstrated good response to chemotherapy, while 7 of 11 (64%) P16 negative tumors demonstrated poor response (P=0.001). Among P16 negative tumors by IHC, *CDKNA2* homozygous gene deletion was present in only 1 case (14%), while the remainder had intact *CDKNA2* or, in two cases, a gain, and LOH (loss of heterozygosity). Although homozygous Rb1 deletion was identified in 15 cases (65%), there was no difference in the prevalence of Rb1 gene deletions between good (8/12) and poor responders (7/11) to chemotherapy (67% vs. 64%, P=1.0).

Conclusions: Although loss of P16 expression in OS is associated with resistance to chemotherapy, only a minority of cases result from deletion of the *CDKNA2* gene. Thus, other mechanisms such as smaller gene mutations or altered transcription, translation, or post-translational modification likely account for loss of expression. Although *Rb1* gene deletions are common in OS and are thought to play an oncogenic role, they are not associated with response or resistance to neoadjuvant chemotherapy.

23 C-MYC Protein Expression by Immunohistochemistry in CIC-DUX4 Rearranged Sarcomas Is Associated with Trisomy 8

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Background: Sarcoma with *CIC-DUX4* fusion is a recently described novel subtype of undifferentiated round cell sarcoma of children and young adults. Additional molecular events occurring in these sarcomas are unknown. Two previously published cases reported trisomy 8, and the molecular analysis of one case suggested that an increase in chromosome 8 copy number might drive c-MYC overexpression in these tumors. To further evaluate this potential relationship, we tested six additional cases of *CIC-DUX4* sarcomas, including two cases with available karyotype, for c-MYC protein expression by immunohistochemistry.

Design: Six formalin-fixed paraffin embedded cases of *EWSR*-negative by FISH, *CIC-DUX4* rearranged sarcomas were tested for c-MYC expression by immunohistochemistry (rabbit monoclonal Y69, Epitomics, CA, USA). Conventional cytogenetics analysis was performed on two cases. In addition, 5 of 6 cases were stained for ERG as we recently encountered an ERG-immunoreactive *CIC-DUX4* tumor which was initially mistaken for a Ewing sarcoma.

Results: The study included 6 patients (5F, 1M, age 20-43), with trunk (2/6) or extremity-based (4/6) tumors. Follow up available in four patients (range 10.2-16.8 mo, mean 13.8 mo) revealed that all four patients developed metastases and died of disease. All six *CIC-DUX4* rearranged sarcomas showed moderate to strong diffuse nuclear c-MYC immunoreactivity, including two cases with trisomy 8. ERG also showed strong diffuse nuclear staining in 3 of 5 cases.

Conclusions: Our results further support trisomy 8 as a recurrent chromosomal abnormality in *CIC-DUX4* sarcomas and strengthen the evidence that chromosome 8 copy number increase may lead to c-MYC overexpression in these tumors. The strong expression of ERG in a proportion of these tumors may lead to diagnostic confusion but the biologic significance of this finding is not known.

24 Favorable Outcome of Early Malignant Peripheral Nerve Sheath Tumor (MPNST) Arising in Neurofibroma

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Background: Early MPNST arising in a neurofibroma (NF) is typically diagnosed based on proposed recommendations including hypercellularity, fascicles, nuclear enlargement, diffuse cytologic atypia and mitoses. Little data is available to determine if these transitioning tumors behave in the same manner as conventional MPNSTs.

Design: In this preliminary study blinded to outcomes, we histologically re-evaluated a panel of nerve sheath tumors previously diagnosed as early MPNST arising in a NF (eMNF) along with a control group of deep-seated NFs. We evaluated hypercellularity, hypocellular (<50%) and hypercellular (>50% cell to stroma ratio) fascicles, nuclear enlargement (nuclei 3x the size of neurofibroma nuclei), diffuse cytologic atypia, coarse chromatin, necrosis and mitoses. The extent of MPNST changes relative to the background NF was estimated. Only cases with a clearly identifiable NF-eMNF interface were included. Isolated hypercellularity or cytologic atypia was not considered sufficient for the diagnosis of eMNF. Cases easily recognized as morphologically high grade MPNST were excluded. Clinical follow-up was obtained.

Results: 11 eMNFs and 11 deep-seated NFs were compared. There was a history of neurofibromatosis in 7 eMNFs and 5 NFs. The following observations were recorded in the eMNF group: increased cellularity (11), hypocellular fascicles (10), hypercellular fascicles (9), nuclear enlargement (4), coarse chromatin (8), necrosis (3), mitoses (8), range 1 to 30 per hpf. Changes suggestive of eMNF comprised from 8 to 80% (mean, 44%) of the background NF. Mean clinical follow up was 34.6 months (range, 9-69 mo). Only two patients died of MPNST, both from eMNFs showing hypercellular fascicles, necrosis, coarse chromatin, mitotic figures, and in one case, diffuse cytologic atypia. All other patients were alive and none had recurrence or metastasis. Deep-seated NFs showed increased cellularity (6), rare hypocellular fascicles (2), scattered cytologic atypia (2) and nuclear enlargement (1) but not hypercellular fascicles, mitotic figures, necrosis or diffuse cytologic atypia.

Conclusions: Historically, patients with MPNST have a 30-60% five year overall survival. Although this is a retrospective analysis with a limited number of cases, it indicates a favorable outcome in most patients with eMNF. These data suggest that the malignant potential of eMNFs may be less than classical MPNST, and larger scale studies are necessary to determine whether these lesions warrant classification as MPNST or whether a classification such as nerve sheath tumor of uncertain malignant potential might be more appropriate.

25 A Novel Superficial CD34-Positive Fibroblastic Sarcoma

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Background: Most fibroblastic sarcomas fall into well-defined clinicopathological groups. We report our experience with a previously unreported fibroblastic sarcoma characterized by superficial location, distinctive histopathological features and CD34 immunoreactivity, which we term "superficial CD34-positive fibroblastic sarcoma".

Design: 19 cases previously coded as "low grade sarcoma, not further classified" and "malignant fibrous histiocytoma, low grade" were retrieved from our consultation archives. Immunohistochemistry (IHC) for CD34, cytokeratins, desmin, Fli1, SMARCB1 and S-100 protein was performed. Because of some similarity to myxoinflammatory fibroblastic sarcoma (MIFS) and pleomorphic hyalinizing angiectatic tumor (PHAT), 5 cases were analyzed by fluoresence *in situ* hybridization for rearrangements of *TGFBR3* and *MGEA5*.

Results: The tumors presented in 10 males and 9 females (mean age 40 years, range 20-76 years) as slowly growing masses of variable size (mean 4.2cm, range 1.5-10cm) confined to the superficial soft tissues of the thigh (6/19); arm, knee, groin (2 each); and neck, shoulder, hip, vulva, buttock, leg and foot (1 each). The tumors were composed of fascicles and sheets of spindled cells with abundant granular, fibrillar or "glassy" cytoplasm. They exhibited marked nuclear pleomorphism, out of proportion to the low mitotic rate (mean < 1/20 HPF). A prominent inflammatory infiltrate was present in all cases. Necrosis was present in one case. Ectatic vessels, stromal and perivascular hyalinization, and myxoid zones with pseudolipoblasts were absent. Tumors were diffusely CD34 positive (19/19), focally positive for keratin (11/16), and negative for other markers. All retained expression of SMARCB1. FISH for *TGFBR3* and/or *MGEA5* rearrangements was negative. Follow up (7 patients, median 5 months, range 2-96 months) revealed no local recurrences or distant metastases. One patient developed regional lymph node metastases following incomplete excision. All patients are currently alive.

Conclusions: Superficial CD-34 fibroblastic sarcoma is a unique low grade sarcoma. Its significance resides in the fact that it most likely has been confused with superficial undifferentiated pleomorphic sarcoma in the past but can be distinguished on the basis of distinctive histopathological features. The clinical, morphological and molecular genetic features of this tumor also differ from those of MIFS and PHAT. Limited follow up suggests it behaves as a low-grade sarcoma with limited potential for lymph node metastases.

26 Evaluation of *GNAS* Mutations in Fibrous Dysplasia and Its Malignant Mimics, Including the Novel Finding of *GNAS* Mutation in Parosteal Osteosarcoma

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Background: Fibrous dysplasia is a benign fibro-osseous disorder that shares morphologic features with several benign and malignant primary bone neoplasms. While the diagnosis of fibrous dysplasia may be straightforward when adequate sampling is coupled with radiologic correlation, pathologists are often asked to evaluate small biopsy specimens. Mutations in *GNAS*, the gene encoding the stimulatory alpha subunit of the heterotrimeric G protein complex, are commonly found in fibrous dysplasia, but very few studies have evaluated *GNAS* mutational status in malignant bone-forming tumors that may simulate fibrous dysplasia. We sought to evaluate the role of *GNAS* mutational analysis as an ancillary technique in distinguishing fibrous dysplasia from its benign and malignant histologic mimics.

Design: Cases of monostotic fibrous dysplasia (N=12), adamantinoma (N=3), osteofibrous dysplasia (N=4), parosteal osteosarcoma (N=7) and low grade central osteosarcoma (N=1) were retrieved from our institutional archives. Clinical, radiologic and histologic features were re-evaluated to confirm the previous diagnoses. DNA was extracted from paraffin-embedded tissues, and *GNAS* exons 8 (region containing codon 201) and 9 (region containing codon 227) were evaluated by PCR and direct sequencing.

Results: Ten cases of fibrous dysplasia harbored *GNAS* mutations in codon 201 (exon 8), including R201H (5 cases) and R201C (5 cases), while two cases had wild type *GNAS*. Four (of 7) parosteal osteosarcomas (humerus (N=2) and femur (N=2); 3 females

and 1 male; age range 15-42 years old) and one low-grade central osteosarcoma (tibia, female, 29 years old) harbored *GNAS* R201C mutations. No *GNAS* codon 227 (exon 9) mutations were found. Three cases of parosteal osteosarcoma had wild type *GNAS*. No *GNAS* mutations were detected in cases of adamantinoma or osteofibrous dysplasia.
Conclusions: In our series, ten of twelve fibrous dysplasia cases had activating *GNAS* mutations, comparable to the reported rates in the literature. However, in our series of seven parosteal osteosarcomas, four cases had *GNAS (*R201C) mutations as did one case of low grade central osteosarcoma. To the best of our knowledge, this is the first report of *GNAS* mutations in parosteal osteosarcoma. Thus. while *GNAS* mutational analysis may help distinguish fibrous dysplasia from benign histologic mimics such as osteofibrous dysplasia and adamantinoma, it may be of limited utility in distinguishing fibrous dysplasia from low grade central and parosteal osteosarcoma.

27 *Isocitrate Dehydrogenase (IDH1/IDH2)* Mutation Types and Frequency in Periosteal Cartilaginous Tumors

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Background: Primary cartilaginous tumors arising on the surface of bone include benign periosteal chondromas and their malignant counterparts, periosteal chondrosarcomas. Recently, somatic mutations in *isocitrate dehydrogenase* (*IDH1*/*IDH2*), encoding an enzyme in the tricarboxylic acid cycle, have been reported in cartilaginous tumors. However, the rarity of periosteal cartilaginous tumors, particularly periosteal chondrosarcomas, has limited the evaluation of *IDH* mutational status in these tumors. We evaluated *IDH* mutational types and frequency in a series of periosteal cartilaginous tumors.
Design: Cases of periosteal chondromas (N=9) and periosteal chondrosarcomas (N=6) were retrieved from our institutional archives. Histologic slides and radiologic images (when available) or reports were reviewed. DNA was extracted from paraffin-embedded tumor tissue and *IDH1* (codon 132) and *IDH2* (codon 172) were evaluated by PCR and pyrosequencing and/or direct sequencing.
Results: Periosteal chondromas presented in 5 males and 4 females (median age 29 years, range 4-51 years) as small masses (mean 2.1 ± 0.9 cm). Among them, seven (78%) had heterozygous mutations in *IDH1* (phalanges N=3, humerus N=2, calcaneus and fibula, N=1); including the R132C (71%) and R132H (29%) mutation. Mutations in *IDH2* were not detected. Periosteal chondromas with wildtype *IDH* (N=2) occurred in the humerus and tibia. Periosteal chondrosarcomas presented in 3 males and 3 females (median age 43 years, range 15-72 years) as large masses (mean 5.8 ± 2.3cm). Among them, two (33%) had heterozygous mutations in *IDH*, including *IDH1* R132C (spine), and *IDH2* R172G (tibia). Periosteal chondrosarcomas with wildtype *IDH* (67%) occurred in the humerus (2), femur and pubis. There was no association between *IDH* mutational status and patient age, tumor size or location.
Conclusions: In our series, *IDH1* mutations occurred in 78% of periosteal chondromas, comparable to the published literature. However, while *IDH1* R132C was the most frequent mutation type in periosteal chondromas, R132H mutations, not previously reported in this tumor subset, comprised the second largest group. In our series of periosteal chondrosarcomas, the *IDH* mutation rate was 33%, which differs from the 100% mutation rate reported in the few cases in the literature. Also, we report the first case of periosteal chondrosarcoma harboring an *IDH2* (R172G) mutation. Our data comprise the largest series of *IDH* mutational data in perisoteal chondrosarcomas reported to date and contribute to the emerging spectrum of *IDH* mutations in cartilaginous tumors.

28 ALK Expression in Angiomatoid Fibrous Histiocytoma (AFH): A Potential Diagnostic Pitfall

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Background: We recently identified a case of primary pulmonary AFH (*EWSR1*-rearranged) that was misdiagnosed as inflammatory myofibroblastic tumor (IMT) based in part on ALK expression by immunohistochemistry. Prompted by this experience, we evaluated ALK expression in AFH as compared to select spindle cell neoplasms with accompanying lymphoplasmacytic infiltrate.
Design: We studied FFPE sections from 10 AFH (9 of 10 confirmed *EWSR1*-rearranged by FISH) and included 15 IMT and 11 follicular dendritic cell sarcomas (FDC) for comparison. ALK expression was evaluated with 3 different antibody clones: D5F3 (1:100, Cell Signaling Technology), 5A4 (1:40, Novocastra) and ALK-1 (1:200, Dako). ALK IHC positive cases were analyzed with FISH using dual color *ALK* break apart probe kit (Abbott Molecular Vysis) for rearrangement of the *ALK* gene (2p23).
Results: The majority of AFH cases studied were positive for ALK IHC with at least one antibody (8/10 DF53, 6/9 5A4, 1/9 ALK-1), most demonstrating moderate to strong cytoplasmic staining. AFH with positive ALK IHC showed no *ALK* gene rearrangement by FISH (0/8). In select AFH cases, the mean *ALK* gene copy number was 1.6-2.1. 67% of IMT were ALK positive by IHC (10/15 DF53, 8/15 5A4, 7/15 ALK-1). Of these, *ALK* gene rearrangement was demonstrated by FISH in 9/10 IMT. All FDC sarcomas were negative for D5F3 and 5A4.
Conclusions: Our results indicate that ALK expression in AFH is common, particularly with the D5F3 and 5A4 antibodies and enhanced detection systems, and is a potential source of diagnostic confusion with IMT. The genetic mechanism behind ALK expression in AFH is not apparent, although it does not appear to be from *ALK* gene rearrangement or amplification as detected by FISH.

29 Histomorphologic Features Predictive of Survival in Osteosarcoma

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Background: Osteosarcoma shows considerable histomorphologic heterogeneity, and traditional grading schemes have not been universally effective in predicting outcome. Following biopsy confirmation, treatment regimens typically consist of neoadjuvant chemotherapy followed by surgical resection; depending on the response to treatment, chemotherapy may be modified accordingly. The purpose of this study is to examine osteosarcoma for histomorphologic features yielding reliable predictors of clinical outcome.
Design: We conducted a retrospective review for osteosarcoma diagnosed on biopsy (needle core, or curettage). Slides were reviewed and histomorphologic tumour attributes recorded, including: histologic subtype, cell shape, cell size, percent necrosis, mitotic rate, nuclear pleomorphism, nucleoli, percent osteoid, extent of mineralization, lymphovascular invasion, chondroid matrix, and multi-nucleated giant cells. Univariate associations with overall survival (OS) were analyzed by Kaplan-Meier survival analysis and the independent effect of predictors assessed by multivariate Cox proportional hazards regression.
Results: 168 patients with a pathologic diagnosis of osteosarcoma, treatment, and follow-up information at our institution were identified. Median OS was 163 months. By univariate analysis, histologic subtype was predictive of OS ($p = 0.017$), with the chondroblastic and telangectatic variants associated with the worst survival, fibroblastic with the longest OS, and the osteoblastic variant in between. Spindle cell shape ($p = 0.04$) was also associated with increased OS, whereas there was a negative trend with the presence of chondroid ($p = 0.086$), reflecting covariance with histologic subtype. Extensive necrosis (>50%) and histologically identifiable nucleoli were poor prognostic features ($p < 0.001$ and $p = 0.022$ respectively). The multivariate model retained subtype ($p = 0.047$), extensive necrosis ($p = 0.002$, HR 2.56, 95% CI 1.40, 4.67) and histologically identifiable nucleoli ($p = 0.023$, HR 1.87, 95% CI 1.09, 3.21) as significant independent predictors.
Conclusions: Tumour necrosis and prominent nucleoli appear to be independent predictors of poor OS in osteosarcoma and may merit inclusion in grading schemes. In contrast, mitotic rate and nuclear pleomorphism do not appear to be predictive of outcome. In addition, these preliminary results confirm histologic subtypes of osteosarcoma are distinct with respect to clinical behaviour and underlying biology.

30 BRAF Mutation in "Sarcomas": A Possible Method To Detect Melanomas

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Background: BRAF is commonly mutated in melanomas (over 60%), as well as some thyroid and colon carcinomas. BRAF mutation has been anecdotally reported in up to 2% of sarcomas. Immunohistochemically, some poorly-differentiated melanomas lose melanocytic markers and some sarcomas demonstrate no lineage of differentiation. Making a definitive diagnosis of melanoma versus sarcoma in this setting can be challenging. This study addresses the rate of BRAF mutation in patients with sarcomas and in patients with both melanoma and sarcoma.
Design: A clinico-pathologic database was queried for patients with sarcoma, with or without melanoma. SNaPshot mutational analysis was performed on formalin-fixed paraffin-embedded tumor tissue from: 1) 50 patients with sarcoma-only, without melanoma; 2) 13 patients with both sarcoma and melanoma (melanoma tissue was not available for 3 of these patients).
Results: In the sarcoma-only group (n=50), BRAF mutation was not identified. Nine sarcoma-only patients had other molecular aberrations. In the sarcoma-melanoma group (n=13), two sarcomas showed BRAF mutation, both undifferentiated sarcomas. Tissue from the two corresponding melanomas was not available for comparison. Three melanomas showed BRAF mutation but the corresponding sarcomas did not. Three sarcomas and one melanoma showed other molecular aberrations.
Conclusions: Rate of BRAF mutation in sarcoma is low. In patients with sarcoma WITHOUT melanoma, the rate in this study is 0%. Interestingly, BRAF mutation was only identified in sarcomas from patients who also had melanomas. Both sarcomas were high grade, poorly differentiated, did not stain for melanocytic markers, and occurred chronologically after the melanomas. In one case, the melanoma was in the left buttock with left groin metastases; the "sarcoma" was in the left pelvis. In the other case, the melanoma was in the left breast; the "sarcoma" was in the left lung. The presence of BRAF mutation in these tumors raises the possibility that poorly differentiated sarcomatoid malignancies with BRAF mutation may represent melanomas. Further investigation into this topic is warranted. BRAF mutational analysis should be considered in patients with poorly differentiated malignancies (especially those with histories of melanoma), as a positive result may indicate melanocytic differentiation.

31 Prognostic Value of Subclassification of Pleomorphic Soft Tissue Sarcoma (STS): Myogenic Versus Non-Myogenic Differentiation

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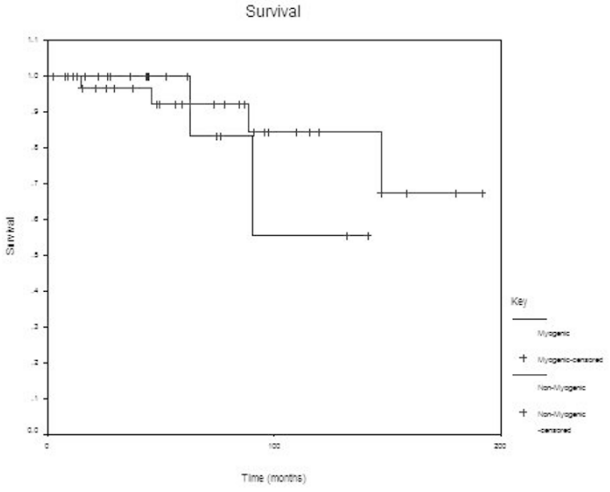
Background: Previous studies have demonstrated that patients with pleomorphic soft tissue sarcoma (STS) with myogenic differentiation have a worse prognosis, but these studies include heterogeneous populations of patients who were not uniformly treated. We sought to determine the prognostic significance of myogenic differentiation in a uniformly treated population of adult patients with high-grade pleomorphic STS of the extremities or trunk at a single institution.

Design: 56 patients with large (≥ 8 cm), grade 2 or 3 extremity or truncal pleomorphic STS received protocol neoadjuvant chemoradiotherapy followed by surgical resection. The tumors were reanalyzed histologically and immunohistochemically and were classified according to strict diagnostic criteria as either non-myogenic or myogenic differentiation if they showed positive staining for at least one muscle marker (muscle actin, smooth muscle actin, desmin). We correlated the line of differentiation with outcome.

Results: Myogenic differentiation was identified in 18 tumors and non-myogenic differentiation was identified in 38 tumors. There were no significant differences between the two groups in tumor size (median 12 cm), grade, or treatment. At a median follow-up of 60 months, the 5-year disease-specific and overall-survival rates in the myogenic and non-myogenic groups were 88% and 88% (p=1.000) and 83% and 79% (p=0.964), respectively.



Conclusions: In a homogeneously treated patients with high-grade extremity and truncal pleomorphic STSwho received neoadjuvant chemoradiotherapy, myogenic differentiation did not predict a worse outcome than non-myogenic differentiation.

32 Radiation Induced Sarcomas Occurring in Desmoid-Type Fibromatosis Are Not Derived from the Primary Tumour

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Background: Desmoid-type fibromatosis (ICD-O 8821/1) is a rare highly infiltrative and locally destructive neoplasm which does not metastasize, but may recur in 19-36% of the cases. They may occur in the context of familial adenomatous polyposis (FAP) which is caused by a mutation in the adenomatous polyposis gene (APC). APC mutations result in activation of the Wnt/β-catenin pathway. An alternative mechanism for activation is a mutation in exon 3 of *CTNNB1*, the gene encoding β-catenin, which results in stabilization of the protein. *CTNNB1* mutations occur in 85% of the sporadic desmoid tumours. These locally aggressive lesions are preferably treated by surgery with a wide margin. If surgery is not possible radiation therapy is often used. In rare cases this may result in the development of a radiation induced sarcomawithin the treatment area. It is unclear whether these sarcomas develop from the primary tumour or arise *de novo* in normal tissue.

Design: To assess this issue we determined whether postradiation sarcomas arising in desmoid-type fibromatosis contain mutations in *CTNNB1*. We have collected four cases and determined the DNA sequence of exon 3 in the desmoid tumour and the subsequent postradiation sarcoma.

Results: In 2 out of 4 cases a T41A activating mutation in *CTNNB1* could only be detected in the desmoid tumor, whereas in the radation induced osteosarcomas developing 6 and 11 years after treatment no *CTNNB1* mutation was detected. A third case showed the S45F hotspot mutation in the original fibromatosis, whereas the postradiation undifferentiated sarcoma developing 6 years later was wildtype for *CTNNB1* exon 3. The fourth case showed the T41A mutation in both in the desmoid fibromatosis as well as in a fibrosarcoma arising within the desmoid tumor.

Conclusions: In conclusion, postradiation sarcomas that occur in the treatment area of desmoid-type fibromatosis preferentially arise *de novo* and are not derived from the original desmoid tumour. Fibrosarcoma arising after this treatment may be derived from the original desmoid fibromatosis.

33 SATB2 Is a Novel Marker of Osteoblastic Differentiation in Bone and Soft Tissue Tumors

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Background: Special AT-rich sequence-binding protein 2 (SATB2) is a nuclear matrix protein that plays a critical role in osteoblast lineage commitment. Targeted knockout of Satb2 in mice results in impaired osteoblast differentiation and craniofacial skeletal defects. Similarly, germline deletions of the *SATB2* locus are a rare cause of craniofacial malformations in humans. Identification of osteosarcomatous differentiation in bone and soft tissue tumors can pose a diagnostic challenge, as osteoid deposition is often limited in extent, and hyalinized collagenous stroma may closely mimic osteoid matrix. We investigated expression of SATB2 by immunohistochemistry (IHC) in osteosarcomas and other bone and soft tissue tumors to evaluate its potential diagnostic utility.

Design: Tissue sections of 61 tumors were evaluated: 27 osteosarcomas [20 of skeletal origin (15 primary tumors and 5 lung metastases), including 14 osteoblastic, 1 chondroblastic, 3 giant cell-rich, 1 parosteal, and 1 periosteal; and 7 extraskeletal], 2 osteoblastomas, 10 chondrosarcomas (5 dedifferentiated, 4 conventional, and 1 mesenchymal), 8 Ewing sarcomas (all confirmed to harbor *EWSR1* rearrangements), 5 dedifferentiated liposarcomas with heterologous osteoblastic differentiation, 6 unclassified pleomorphic sarcomas, and 1 leiomyosarcoma. IHC was performed following pressure cooker antigen retrieval using a polyclonal anti-SATB2 antibody (1:250; Sigma).

Results: All 20 skeletal osteosarcomas, 6 of 7 extraskeletal osteosarcomas, and both osteoblastomas showed nuclear immunoreactivity for SATB2, usually of moderate-to-strong intensity, consistently in tumor cells adjacent to osteoid and more variably in cells not associated with matrix. Focal reactivity was also observed in 3 of 5 dedifferentiated chondrosarcomas, and in all 5 dedifferentiated liposarcomas, only in areas of heterologous osteosarcomatous differentiation. All other tumor types were completely negative for SATB2. Staining intensity was generally higher in small biopsies and lung metastases, compared to bone resection specimens, likely secondary to decalcification.

Conclusions: SATB2 is a specific marker of osteoblastic differentiation in mesenchymal tumors, both osteosarcomas and sarcomas with heterologous differentiation. Nuclear staining for SATB2 may be useful to distinguish osteosarcoma from histologic mimics and other osseous neoplasms.

34 WWTR1-CAMTA1 Fusion Product: A Unique Marker of Epithelioid Hemangioendothelioma

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Background: Epithelioid haemangioendothelioma (EHE) is an endothelial neoplasm exhibiting an unusual morphology, and features that occasionally result in diagnostic confusion. The recent discovery of the WWTR1-CAMTA1 gene fusion in EHE confirmed this to be a distinct entity. The purpose of this project was to perform an institutional review of vascular lesions diagnosed as EHE and to assess the presence and specificity of the WWTR1-CAMTA1 fusion product using formalin-fixed paraffin-embedded tissue.

Design: A retrospective review was performed for recent cases of epithelioid haemangioendothelioma, epithelioid haemangioma (EH) and epithelioid angiosarcoma (EAS) diagnosed at our institution. Two cases each of haemangioma and cutaneous angiosarcoma served as negative controls. Ribonucleic acid (RNA) was extracted from archival formalin-fixed, paraffin-embedded tissue and reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using primers specific for the breakpoint between exon 4 of WWTR1 and exons 8 and 9 of CAMTA1.

Results: A total of 7 EHE, 1 EH and 5 EAS were identified. Three cases of EHE were found to contain the WWTR1-CAMTA1 fusion product, 1 case was negative and 1 was inconclusive. RNA could not be obtained from 3 cases. The negative case was noted to contain unconventional histological features. All EH and EAS cases were negative for the fusion product, as were the hemangioma and angiosarcoma.

Conclusions: We confirm the specificity of the WWTR1-CAMTA1 in the diagnosis of EHE using formalin-fixed paraffin-embedded tissue. While one case was negative, the morphology differed somewhat from conventional EHE raising the possibility of an alternative sub-classification. These findings confirm (i) the diagnostic utility of this PCR-based assay in formalin-fixed paraffin-embedded tissue, and (ii) that epithelioid haemangioendothelioma represents a distinct pathologic entity.

35 MED12 Exon 2 Mutation Analysis in Different Subtypes of Smooth Muscle Tumors Confirms Genetic Heterogeneity

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Background: Recently mutations in exon 2 of the *mediator complex subunit* 12 (MED12) gene have been described in 50-70% of uterine leiomyomas (fibroids); the recurrent nature of these mutations suggests an important role in their pathogenesis. MED12 is involved in regulation of transcription and Wnt-signaling. So far, little is known about the pathogenesis of the different subtypes of extra-uterine leiomyomas and of the malignant counterpart leiomyosarcoma.

Design: We therefore performed mutation analysis using PCR and direct sequencing of exon 2 of MED12 and immunohistochemistry for β-catenin, using 70 tumors of 65 different patients; 19 uterine leiomyomas, 6 abdominal leiomyomas, 5 angioleiomyomas, 5 leiomyomas of soft tissue, 5 piloleiomyomas, 7 uterine leiomyosarcomas and 23 leiomyosarcomas of soft tissue.

Results: In 11 of 19 uterine leiomyomas (56%) a mutation in MED12 was identified, which is consistent with the literature. One of 6 abdominal leiomyomas, of which a primary uterine origin cannot be excluded, displayed a homozygous mutation. Mutations were absent in all other extra-uterine leiomyomas. Of the 30 leiomyosarcomas, only one uterine leiomyosarcoma harbored a mutation. Beta-catenin immunohistochemistry revealed nuclear staining in 10 of 40 leiomyomas and 3 of 30 leiomyosarcomas. Among the mutated uterine leiomyomas, 54% displayed nuclear positivity compared to none of the non-mutated uterine fibroids; and 80% of the piloleiomyomas were positive.

Conclusions: As no MED12 alterations are found in convincing extra-uterine leiomyomas and leiomyosarcomas of soft tissue these tumors probably arise through different pathogenetic mechanisms emphasizing the genetic heterogeneity of smooth muscle tumors. Since only 54% of the MED12 mutated uterine leiomyomas displayed nuclear β-catenin, this suggests that in addition to canonical Wnt-signaling other pathways are responsible for tumorigenesis in MED12 mutated tumors. The β-catenin

pathway might be involved in the development of sporadic piloleiomyomas as 80% showed nuclear β-catenin immunopositivity. Thus, our results confirm genetic heterogeneity and exclude a role for MED12 in soft tissue smooth muscle tumors.

36 MicroRNA Expression Profiling Identifies Potential Prognostic Indicators in Primary Solitary Fibrous Tumors

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Background: The behavior of solitary fibrous tumors is notoriously difficult to predict and reliable biomarkers are lacking. MicroRNA (miRNA) are non-coding RNAs with post-transcriptional regulatory functions, which have been implicated in tumorigenesis of a number of malignancies. Currently, the role of miRNA in primary solitary fibrous tumors (SFT) is poorly understood. We therefore sought to determine the expression patterns and prognostic value of miRNA in benign and malignant primary SFT.

Design: RNA was extracted from 18 formalin-fixed paraffin-embedded primary SFTs: 9 tumors that gave rise to metastases, and 9 tumors that did not. MiRNA expression profiling was performed using a real-time PCR based platform on a Taqman Human miRNA array card containing 378 annotated miRNAs. Expression was normalized to housekeeping miRNAs. Two sample t-tests were used on a probe-by-probe basis to compare malignant with benign tumors.

Results: The benign tumors had a median clinical follow-up of 100 (range 77-147) months, while the malignant tumors metastasized at a median of 30 (range, at presentation – 71) months. Sites included pleura (n=6), abdomen (n=6), and soft tissues of head, extremity, or trunk (n=6). Two sample t-tests between metastasizing and benign populations identified 9 differentially expressed miRNA with p-value $\leq$0.05. MiR-30c, miR-489, miR-886-3p, miR-142-3p, miR-597, mir-34c, miR-570, and miR-653 showed 1.6 to 18.4-fold reduction in expression in malignant SFT vs. benign, while miR-339-5p and miR-450b-5p had 14.7 to 24.9-fold increase in expression in malignant SFT.

Conclusions: We identified a subset of miRNA that differentiated between benign and malignant SFT in formalin fixed paraffin-embedded tissue. Several of these miRNAs have been previously identified as having tumor suppressor functions. In particular, miR-489, miR-30c, miR-34c and miR-886-3p have been shown to be downregulated in carcinogenesis. MiR-886-3p has been postulated to regulate focal adhesion pathways and DNA replication, while miR-489 has been suggested to play a role in cell growth and mesenchymal differentiation, and miR-34c and miR-30c may repress cell migration and invasion. In our population, these were downregulated in aggressive tumors, suggesting that they may function similarly in SFT. Network analysis and biomarker validation is ongoing.

37 Peripheral Hemangioblastoma: Clinicopathologic & Immunohistochemical Analysis of 21 Cases

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Background: Hemangioblastoma is a rare tumor of uncertain histotype that typically arises in the cerebellum, often in the setting of Von Hippel-Lindau syndrome (VHL). Exceptional cases of hemangioblastoma arising outside the central nervous system (CNS) have been reported, but little is known about their clinicopathologic and immunohistochemical features.

Design: 21 cases of hemangioblastoma arising at peripheral sites (PH) were identified in consult files. Clinical, morphologic and immunohistochemical features were evaluated. Outcome data were obtained from referring pathologists.

Results: 11 patients were female and 10 male; median age was 58 years (range 27-79). Tumors arose in spinal nerve roots (12), kidney (3), intestine (2), orbit (1), forearm (1), peritoneum (1) and flank (1). 5 patients had VHL; another 5 had lesions suggestive of VHL. 1 patient had tuberous sclerosis. Median tumor size was 4 cm (range 1.3-15 cm). Most tumors were well circumscribed; 6 were infiltrative-3 extended into bone and 1 into pleura. Tumors had a complex capillary network with larger thin or thick walled vessels in a solid and often lobular growth pattern, similar to CNS hemangioblastoma. In 9 cases the larger vessels showed a branching hemangiopericytoma-like pattern. All tumors had an admixed population of plump spindle cells and microvacuolated cells with pale eosinophilic or clear cytoplasm, which often mimicked lipoblasts or renal cell carcinoma (RCC). In 5 cases the microvacuolated cells were scant. Stromal cell nuclei were hyperchromatic or vesicular with inconspicuous nucleoli. 4 tumors showed marked nuclear pleomorphism. Mitotic activity was low (range <1-2/10 HPF). No necrosis or lymphovascular invasion was identified. Tumor cells expressed inhibin in 95% (19/20), NSE in 79% (15/19), S100 in 63% (12/19), as well as GLUT1 (7/10, mostly weak), SMA (4/5), EMA (2/8, focal), PAX-8 (1/10), aquaporin (3/6) and desmin (1/4). Brachyury was consistently negative (0/19), as were keratin, HMB-45, melanA and GFAP. CD31 and CD34 highlighted tumor vasculature. Follow up information was available for 17 patients (mean 34 months; range 5-117). 1 patient died of metastatic RCC. 3 had locally persistent PH after incomplete resection. Local recurrence or distant metastasis has not been identified in any patient so far.

Conclusions: PH is rare but may mimic some malignant tumors; the distinct immunohistochemical profile can aid diagnosis. Unresectable cases may be locally aggressive, but complete excision appears to be curative. Recognition of this tumor may identify patients in whom testing for VHL is warranted.

38 Do Meningeal Hemangiopericytoma and Mesenchymal Chondrosarcoma Belong to the Same Biologic Spectrum? A Study of *HEY1-NCOA2* Fusion

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Background: Meningeal hemangiopericytoma (meningeal HPC) is an aggressive neoplasm characterized by high rates of local recurrence and extracranial metastases. Although earlier classifications have suggested a relationship to variants of solitary fibrous tumor and meningioma, more recent work has shown that meningeal HPC is a distinct entity with an uncertain pathogenesis. Mesenchymal chondrosarcoma is a sarcoma which may involve both skeletal and extraskeletal sites but has a predilection for the meninges. In addition to similar clinical presentations, both meningeal HPC and mesenchymal chondrosarcoma share overlapping morphologic features, including oval to slightly spindled cells, variable collagen deposition and a branching, "staghorn" vascular pattern. Recently, a novel *HEY1-NCOA2* fusion was reported as a recurrent event in mesenchymal chondrosarcomas. In contrast, no consistent cytogenetic or molecular aberration has been found in meningeal HPC.

Design: Thirteen mesenchymal chondrosarcomas and 18 meningeal HPCs were identified at the Mayo Clinic and Cleveland Clinic surgical pathology archives. RNA was extracted from paraffin-embedded tissues, and the tumors were evaluated for *HEY1-NCOA2* fusion by RT-PCR. Controls included three chondroblastomas, two chondromyxoid fibromas, five Ewing sarcomas and five synovial sarcomas.

Results: *HEY1-NCOA2* fusion transcript was detected in 6/6 cases of mesenchymal chondrosarcoma but in none of the meningeal HPC cases (0/11). In 7 cases each of mesenchymal chondrosarcoma and meningeal HPC, RT-PCR was uninformative due to RNA degradation. All controls were negative for *HEY1-NCOA2* fusion transcript.

Conclusions: Although there is significant clinical and histologic overlap between mesenchymal chondrosarcoma and meningeal hemangiopericytoma, these results show (1) that they are distinct at the molecular level and (2) that the identification of *HEY1-NCOA2* can be used as an auxiliary diagnostic tool to differentiate these entities.

39 Utility of p16 in Distinguishing Lipomas and Well-Differentiated Liposarcomas Compared to Fluorescence In-Situ Hybridization

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Background: Fluorescence In-Situ Hybridization (FISH) for amplification of *MDM2* is considered the gold standard assay for the diagnosis of well-differentiated liposarcoma (WDL). It is especially useful in lipoma-like WDL, in which cytologic atypia may be focal. Immunohistochemistry (IHC) for p16 has recently been suggested as a sensitive and specific method for distinguishing these two entities, but it has not been validated on cases in which the diagnosis was confirmed by FISH analysis.

Design: A series of 19 lipoma-like WDL and 21 lipomas in which the diagnosis had been previously confirmed by FISH analysis were stained for p16 by IHC. Nuclear p16 staining was scored according to previously published methods: 0% of cells staining was recorded as negative, 1-10% as focal, 11%-50% as multifocal, and >50% as diffuse. The presence of strong, diffuse cytoplasmic p16 staining was also recorded. Regions of fat necrosis were excluded from evaluation.

Results: Nuclear p16 staining was significantly increased in WDL compared to lipoma (Mann-Whitney, P=<0.0001). Of the 19 WDL, 17 (89%) showed at least multifocal strong staining. In contrast, 18 of 21 lipomas (86%) showed no more than focal strong staining for p16; 3 cases showed multifocal strong staining. ROC analysis indicated maximum diagnostic accuracy (88%) was achieved using multifocal strong staining as the threshold value for defining a positive IHC result. Diagnostic performance parameters using this criterion for nuclear p16 in the diagnosis of WDL are presented in the table below.

Sensitivity (95% CI)	0.90 (0.67-0.99)
Specificity (95% CI)	0.86 (0.64-0.97)
Positive likelihood ratio (95% CI)	6.26 (2.17-18.10)
Negative likelihood ratio (95% CI)	0.12 (0.03-0.46)

Although cytoplasmic staining was specific for WDL (95%), it lacked diagnostic sensitivity (53%).

Conclusions: Multifocal or diffuse strong nuclear staining for p16 in a well-differentiated lipomatous neoplasm is highly suggestive of malignancy. Strong nuclear staining limited to $\leq$10% of adipocytes may be seen routinely in lipoma. Although nuclear staining for p16 has been described in lipomas with fat necrosis, we report for the first time multifocal strong p16 staining of adipocyte nuclei within viable regions of lipoma (3/21 cases). While there was not complete concordance (0.88; 95% CI 0.73-0.96) between FISH results and nuclear p16 staining in WDL, the IHC assay performs well. For situations where turn-around time and/or cost are priorities, IHC for p16 may offer an alternative to FISH in the differential diagnosis of well-differentiated lipomatous neoplasms.

40 *PHF1* Rearrangements in Ossifying Fibromyxoid Tumors (OFMT), with Emphasis on the Malignant Variant

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Background: OFMT is a rare borderline malignant mesenchymal tumor of uncertain lineage. Very recently, Gebre-Medhin and colleagues have reported rearrangements of the endometrial stromal sarcoma-associated gene *PHF1* in subsets of OFMT, including typical, atypical and malignant variants (Am J Pathol 2012; 181; 1069). We sought to confirm and extend these findings, in particular with regards to malignant OFMT, the existence of which has been debated.

Design: All available slides from 11 OFMT were retrieved from our archives and classifed according to the Folpe and Weiss (2003) OFMT classification scheme. Interphase FISH was performed on paraffin-embedded sections using a clinically validated, laboratory developed break apart probe for detection of rearrangements of the *PHF1* gene according to an established protocol.
Results: The tumors involved 7 men and 4 women, with a median age of 54 years (20-83 years), and arose in the soft tissues of the head and neck (n=2), trunk (n=1), upper (n=2) and lower limbs (n=6). OFMT were classified as typical (3 cases), atypical (1 case) and malignant (7 cases). *PHF1* rearrangements were identified in 6/11 (55%) cases including 1/3 (33%) typical, 1/1 (100%) atypical and 4/6 (66%) malignant cases.
Conclusions: We have confirmed *PHF1* rearrangements as frequent molecular cytogenetic findings in OFMT of all types. We conclude that the frequency of *PHF1* gene rearrangements in OFMT; 1) imply a pathogenetic role, 2) emphatically confirm the existence of malignant OFMT as part of the histologic spectrum of a single entity, and 3) and underscore the utilitiy of molecular genetic analysis in the diagnosis of malignant or a histologically ambiguous cases of OFMT. On-going molecular genetic study of a larger number of OFMT of all types should help to clarify the relative frequency of *PHF1* rearrangements in these rare mesenchymal tumors.

41 NY-ESO-1 Is a Sensitive and Specific Marker for Myxoid and Round Cell Liposarcomas among Other Mesenchymal Myxoid Neoplasms

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Background: Myxoid and round cell liposarcomas (MRCL) constitute approximately one-third of all liposarcomas, a relatively common group of fat-derived soft tissue sarcomas. The histomorphology is described as a continuum between a highly differentiated myxoid component and a poorly differentiated round cell component. The gold standard of diagnosis is dependent on classic histomorphology and/or identification of t(12;16)(q13;p11) translocation by conventional cytogenetics or demonstration of *CHOP (DDIT3)* gene rearrangements by fluorescent in situ hybridization. There are currently no immunohistochemical (IHC) stains available that are diagnostic of MRCL. The broad range of myxoid neoplasms includes a variety of sarcomas, many of which can show striking morphologic mimicry with MRCL. Given the notable differences in disease biology among myxoid neoplasms, which range from benign to aggressive, an accurate diagnosis is imperative for proper treatment and prognostication. Prompted by our recent study showing frequent expression of the cancer-testis (CT) antigen NY-ESO-1 in MRCL, we sought to evaluate the utility of NY-ESO-1 as an IHC marker for MRCL among mesenchymal myxoid neoplasm within the differential diagnosis.
Design: Formalin-fixed, paraffin-embedded block was obtained for the following mesenchymal myxoid neoplasms (n=107): MRCL (n=39); extra-cardiac myxoma (n=39); extraskeletal myxoid chondrosarcoma (n=12); myxofibrosarcoma (n=10: 5 low grade, 2 intermediate grade, 3 high grade); and low grade fibromyxoid sarcoma (n=7). Utilizing standard IHC staining protocols, full sections were stained with NY-ESO-1 (clone E978; Santa Cruz Biotechnology). Staining was assessed for intensity (1-2+), percentage of tumor positivity, and location.
Results: 35/39 (90%) of the MRCL demonstrated positive NY-ESO-1 staining. The majority of the positive cases (33/35; 94%) showed strong, homogenous staining (>50% tumor positivity). Two cases (6%) showed weak (1+ intensity), patchy immunoreactivity (20-30% tumor positivity). Immunoreactivity was predominantly cytoplasmic with some nuclear staining. All the other neoplasms evaluated were negative for NY-ESO-1.
Conclusions: NY-ESO-1 appears to be a sensitive and specific IHC marker for MRCL among mesenchymal myxoid neoplasms. The assessment of NY-ESO-1 expression by IHC in the appropriate setting provides a cheaper, faster, and more accessible confirmatory test especially in a low resource setting.

42 Perivascular Stem Cells Induce Bone formation and Vasculogenesis in Ectopic and Bone Injury Models

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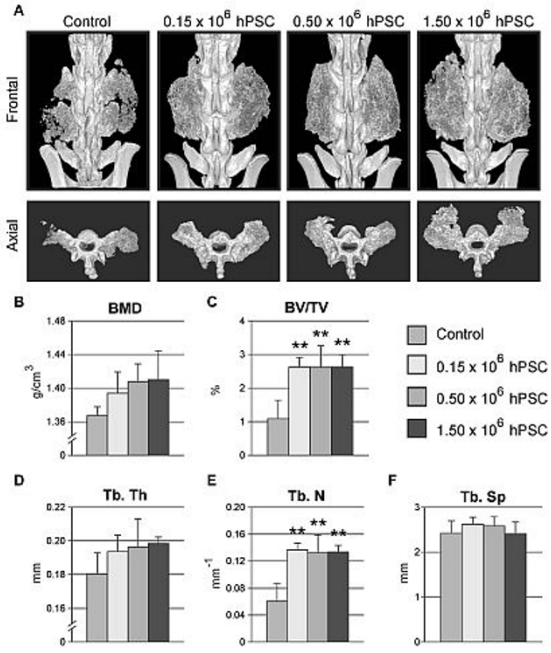
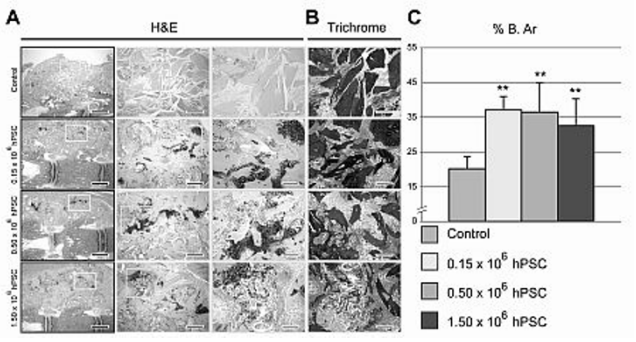
Background: Perivascular stem cells (PSCs) have recently been identified as phenotypically identical to mesenchymal stem cell (MSCs). This new insight has led to interest in the prospective identification of PSCs for the engineering of bone and soft tissue. Here, we describe the frequency of PSCs within adipose tissue, and the tissue morphology and functional outcomes after *in vivo* PSC application. Overall, we identify PSCs as a purified MSC population for bone tissue engineering.
Design: PSCs were obtained from human adipose tissue from than N>70 donors, based on expression of CD34 and CD146. PSCs were compared to an unsorted, patient-matched stromal population (stromal vascular fraction, SVF). Two methods were used to compare SVF to PSCs. First, an ectopic model of bone formation, using mouse intramuscular implantation. Second, rat posterolateral spine fusion was performed. Equivalent numbers of SVF or PSCs were used. *In vivo* analyses included micro CT, cell tracking, histology and immunohistochemistry (markers of osteogenesis, vasculogenesis, and proliferation), and biomechanical analyses.
Results: PSCs represent an abundant portion of adipose tissue (43% of SVF), with multilineage potential. *In vivo*, PSCs showed robust ectopic OPN+, OCN+ bone formation, and increased vasculogenesis. In a spinal fusion model, unsorted SVF did not induce fusion. In contrast, PSCs induced 80-100% fusion. This was accompanied by radiographic and histologic evidence of endochondral ossification.





Conclusions: PSCs are a newly recognized MSC population, harvestable from any vascularized organ. PSCs have robust osteogenic properties and hold promise for future efforts in skeletal tissue regeneration.

43 Sclerosing Paratesticular Rhabdomyoma: A Morphologically Distinct Variant

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Background: Rhabdomyomas, currently classified into fetal, adult, and genital types, are rare. Fetal and adult types typically present in the head and neck, while the genital type occurs usually in the vagina or cervix. We describe a distinct morphologic variant of rhabdomyoma which affects young men in the paratesticular region.
Design: Four cases identified between 2006 and 2011 were retrieved from consultation files. H&E and immunohistochemical stains were examined. Clinical and follow-up information was obtained from referring pathologists.
Results: The four males were aged 19, 24, 27, and 42 years (median 25.5 years). All tumors arose in paratesticular soft tissue (two left, two right); none involved testicular parenchyma. The median tumor size was 5.75 cm (range 3.5-12 cm). Grossly, tumors were well-circumscribed and had uniform tan-white cut surfaces. Microscopically, tumors were characterized by bundles of large well-differentiated rhabdomyoblasts, with copious eosinophilic cytoplasm, which were round, polygonal, and occasionally strap-shaped. The rhabdomyoblasts were set in a dense hyalinized collagenous stroma. Dense lymphoplasmacytic aggregates were present in the stroma. Tumor cells had round, occasionally vesicular, nuclei (sometimes binucleate or multinucleate) with small or inconspicuous nucleoli. All tumors lacked nuclear atypia and necrosis. Mitotic activity was virtually absent, though one tumor showed a count of 1 per 50 HPF. All tumors were diffusely positive for desmin. Three tumors were diffusely positive for fast myosin and the fourth was positive for myf-4. MDM2 (0/2), CDK4 (0/1), S-100 (0/2), GFAP (0/1), and SMA (0/1) immunohistochemistry was negative. All patients were treated by local excision (three with positive margins). Three patients with known follow-up had no evidence of tumor recurrence or disease progression (median follow-up time 5 months).

Conclusions: Sclerosing rhabdomyoma appears to be a morphologically distinct variant, affecting primarily younger male patients in the paratesticular soft tissues. Of date, only 3 similar cases appear to have been reported as single case reports. The clinical course as determined thus far is benign, similar to other rhabdomyoma types (which may occasionally show local recurrence secondary to incomplete excision).

44 Outpatient Core Needle Biopsy Versus Intraoperative Open Surgical Biopsy in the Diagnosis and Grading of Soft Tissue Neoplasms: Is One Method Better Than the Other?

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Background: Soft tissue neoplasms are notorious for their morphologic heterogeneity, not only from one specimen to another but also within an individual specimen. Although the open surgical biopsy (OSB) is a mainstay for diagnosis of these tumors, surgeons are more frequently utilizing the core needle biopsy in the outpatient setting (OCNB) due to its convenience and lower risk of morbidity to the patient. The aim of our study was to evaluate and compare the accuracy of OSB and OCNB in the diagnosis and grading of soft tissue neoplasms.

Design: Twenty-five (25) OCNBs and 76 OSBs of soft tissue neoplasms from 2010 to the present were collected from our archives. Each biopsy included a follow-up resection specimen. Biopsies and corresponding subsequent resections were evaluated by two pathologists (one with specific expertise in soft tissue pathology) for the determination of adequate sampling, tumor grading (benign, low grade malignant, high grade malignant), and diagnosis (if possible). The tissue of origin (e.g., adipocytic, etc) was also recorded in order to assess whether tumors of a particular origin were more or less acurately diagnosed or graded on biopsy.

Results: Twenty of twenty-five (80%) OCNBs were deemed representative of the subsequent resections. The 5 non-representative cases related to a change in grade from benign to low grade malignant (2 well differentiated liposarcomas and 1 low-grade fibromyxoid sarcoma) or a change in diagnosis (2 pleomorphic liposarcomas). Sixty-four of 76 (84%) OSBs were deemed representative. Three of the 4 non-representative cases related to a change in grade from benign to low grade malignant (all well differentiated liposarcomas), and one resulted in a change in diagnosis but no change in grade (foamy macrophages to pigmented villonodular synovitis). The difference in histologic accuracy between OCNB and OSB, 80% and 84% respectively, was not statistically significant (P=0.3099).

Conclusions: There was no significant difference in representative sampling, diagnosis, or grading between OCNB and OSB in this study. Given the convenience of the procedure and the lower risk of morbidity, OCNB appears to be a viable and favorable alternative to OSB in the diagnosis and grading of soft tissue neoplasms; however, care should be taken with adipocytic tumors, particularly well differentiated lipomatous neoplasms, as diagnostic elements of malignancy are less likely to be present in small specimens than for tumors of other origins.

45 Utility of Sox-10 To Distinguish MPNST from Synovial Sarcoma with a Focus on Intraneural Synovial Sarcoma

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Background: Synovial sarcoma (SS) is a spindle cell tumor of unknown histotype, characterized by a reproducible t(X;18) rearrangement. Monophasic SS and malignant peripheral nerve sheath tumor (MPNST) are particularly difficult to distinguish given similar histomorphology. That SS can present intraneurally, or express S-100 protein, further blurs the distinction between these tumors. Although genetic confirmation of t(X;18) is a useful adjunct to diagnosis of such difficult cases, immunohistochemistry is more rapid and cost effective at many institutions. The Sox-10 transcription factor, a putative marker of neural crest differentiation may have diagnostic utility in this differential but immunohistochemical data are limited.

Design: Ninety-seven cases of MPNST and 97 cases of SS were retrospectively identified from the archives of UCSF Medical Center and Mayo Clinic. All SS were genetically confirmed for the presence of t(X;18) by fluorescence in-situ hybridization and/or SYT rearrangements by RT-PCR. Four SS were intraneural and 69 were monophasic. Immunohistochemical staining using standard methods for Sox-10 was performed and evaluated for nuclear positivity independently by two pathologists who were blinded to diagnosis. The intensity was scored semi-quantitatively (score 0 to 3+) accounting for both the fraction of cells staining and staining intensity.

Results: Six of 97 (6%) SS were positive for Sox-10 whereas 53/97 (54%) MPNST were positive. Most of the Sox-10 positive MPNST (n=39, 74%) showed 2+ or 3+ staining. Importantly, however, two of the intraneural SS showed 2+ Sox-10 staining, in clusters of spindle cells. The sensitivity and specificity of Sox-10 for the diagnosis of MPNST over SS were 55% and 93%, respectively with a positive predictive value of 90% and negative predictive value of 67%.

Conclusions: Nuclear Sox-10 staining is present in the majority of MPNST, consistent with the neural crest origin of these tumors. In contrast, nuclear Sox-10 staining is rarely seen in SS. Sox-10 can be a useful and specific, albeit not very sensitive, immunohistochemical test for supporting a diagnosis of MPNST over SS. However, the stain needs to be interpreted with caution in intraneural tumors, since intraneural SS are commonly positive. It remains to be determined whether Sox-10 positive cells in intraneural SS represent entrapped Schwann cells, Sox-10 staining of synovial sarcoma cells, or both.

46 Angiomatoid Fibrous Histiocytoma: Clincopathological and Molecular Characterization with Emphasis on Variant Histomorphology

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Background: Angiomatoid fibrous histiocytoma (AFH) is typified by whirls of plump spindle cells with a triad of pseudoangiomatoid spaces, fibrous pseudocapsules, and lymphocytic cuffs. However, hallmark fusion genes in classical AFH are insufficiently characterized in variant cases.

Design: Thirteen AFHs, including 11 with confirmed hallmark translocation, were reappraised for classic features, reactive osteoclasts, mitotic rates, and stromal, architectural, and cytomorphological variations, with CD99, desmin, and EMA stained in available cases.

Results: Seven males and 6 females aged from 4 to 63 (median, 13) years, including 3 older than 30 years, had AFHs affecting the extremities (n=6), trunk (n=4), and scalp (n=3). Four showed solid histology devoid of pseudoangiomatoid spaces and another one lacked peripheral lymphoid infiltrates, while fibrous pseudocapsules were observed in each case. Nuclear pleomorphism was striking in 2 cases, moderate in 7, and absent in 4, with osteocalsts seen in 2 cases. In 3 AFHs with sclerotic stroma, one exhibited perivascular hyalinization and nuclear palisading, reminiscent of a schwannoma. In 3 varyingly myxoid tumors, one displayed prominent reticular arrangement of spindle cells in abundant myxoid stroma, resembling a myoepithelioma. Apart from split *EWSR1* FISH signals in 4 cases tested, *EWSR1-ATF1* fusion was detected in a myoepithelioma-like AFH and *EWSR1-CREB1* in another 9, including a schwannoma-like case. Immunohistochemically, 56% of AFHs expressed EMA, 78% desmin, and 100% CD99.

Conclusions: Molecular testing can aid in diagnosis of variant AFHs in an awareness of their diverse clinicopathological features.

47 Chondroid Chordoma: A Chordoma Subtype with Worse Prognosis

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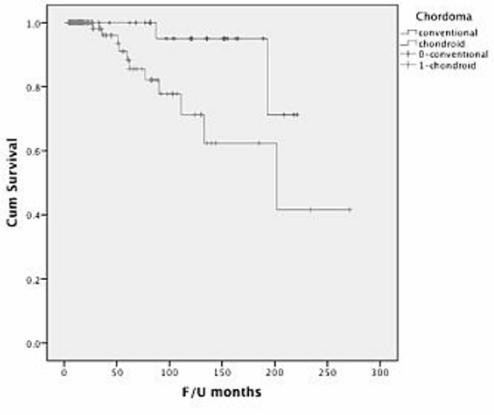
Background: Chordoma is a rare tumor that accounts for 1-4% of primary malignant bone tumors and usually arises in the axial skeleton. Chondroid chordomas occur almost exclusively at the base of the skull and demonstrate morphologic features of chordoma with chondroid areas mimicking cartilaginous neoplasms. Some sources indicate a better prognosis for this subtype of chordoma, but the finding is not universal and studies have included relatively small numbers of chondroid chordomas for assessment.

Design: We aimed to compare the prognosis of chondroid and conventional chordomas of the clival region, the most common location for chondroid chordomas. Our pathology database contained 273 patients with chondroid chordomas from 1990 to the present. 109 of these patients had both clival tumors and available clinical follow up. We compared this cohort to a group of 32 clival conventional chordomas chosen on availability of clinical follow up. Clinicopathologic features and prognosis were compared using logrank tests and Kaplan-Meier curves.

Results: Chondroid chordoma patients were 44% female and 37.2 years on average; patients with conventional chordomas were 47% female and averaged 37.4 years. Differences in age and gender were non-significant (p = 0.49 and 0.46, respectively). Patients with chondroid chordomas had a poorer overall mean survival than those with conventional chordomas (187.4 vs. 207.7 months, p = 0.02) as shown in Figure 1. Additionally patients with chondroid chordomas had a shorter disease-free mean survival than those with conventional chordomas (57.4 vs. 127.4 months, p = 0.001).

Figure 1: Overall Survival of Chondroid Chordomas vs. Conventional Chordomas of the Clivus



Conclusions: Chondroid chordomas of the clivus appear to exhibit a poorer prognosis than clival conventional chordomas. Our results are in contrast to historical reports of chondroid chordomas with a better than or similar prognosis to conventional types. To our knowledge this study represents the largest cohort of chondroid chordomas analyzed. Additional studies are underway to assess for possible contributing factors and increase the size of the comparison cohort. While these findings should be confirmed with further research, our experience indicates that chondroid chordomas may represent a distinct biological subset of chordomas with a more aggressive behavior.

48 Mitotic Count Is Sufficient To Predict Prognosis for Extremity Spindle Cell Soft Tissue Sarcomas

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Background: The grade of spindle cell sarcomas is considered one of the most important predictors of prognosis. Tumor type (differentiation), mitotic count and tumor necrosis are the parameters used in the French Federation of Cancer Centers Sarcoma Group (FNCLCC) grading system. The objective of this study was to determine which of these histological features have prognostic significance with the goal to simplify the grading system.

Design: 107 incisional biopsies of non-metastatic primary spindle cell sarcomas of the extremities were retrieved from our archives. All cases had a minimum of two year follow-up. Patient data and outcome, and tumor characteristics were recorded. Tumors were reviewed and evaluated according to the FNCLCC grading system. The prognostic significance of mitotic count, tumor necrosis, size and depth were evaluated. Kaplan-Meier survival curves were generated to correlate the grade and each of these parameters with metastases and disease-free survivals for univariate analysis. Independent effect of predictors was assessed by multivariate Cox proportional hazards regression.

Results: There were 52 male and 55 female patients with a mean age of 56 years and an average tumor size of 8 cm. 57% of tumors were deep and 43% were superficial. There was a wide spectrum of tumor types. The percentages of grades 1, 2 and 3 tumors were 10%, 44% and 48%, respectively. Both tumor size (<5 or ≥5cm) and depth (superficial vs deep) were predictive of outcome (eg. metastasis-free survival: p=0.0001 and p=0.006 respectively) confirming that the tumor population was behaving as expected. FNCLCC grading was predictive of outcome (disease-free and metastasis-free) as were the individual parameters of the FNCLCC for necrosis and mitoses. When these parameters were simplified to a mitotic count of either <10 mitoses or ≥10 mitoses /1.7mm^2 and necrosis as either present or absent, they were predictive of both metastasis and disease-free survivals by univariate analysis (Mitotic count: p=0.0001; p=0.001 and Necrosis: p=0.013, and p=0.007, respectively). Multivariate analysis suggested that mitotic count was the most significant histological factor in predicting prognosis (p=0.001). Necrosis was not predictive in this analysis.

Conclusions: This data suggests that assessing the mitotic count (<10 or ≥ 10 mitoses /1.7mm^2) only may be sufficient to predict prognosis in incisional biopsies of spindle cell extremity sarcomas. A larger prospective study is required to confirm this observation and assess observer reproducibility.

49 Akt-mTOR Pathway Activation Analysis in Re-Classified Pediatric SMARCB1/INI1-Deficient Tumor

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Background: Pediatric INI1-deficient tumor including unclassified sarcoma shows a highly aggressive biological behavior and poor prognosis. The reason for such dismal prognosis is due to a poor response for well-known antitumor drugs and difficulty in diagnosis. Therefore, an investigation of novel antitumor drugs and re-classification of pediatric INI1-deficient tumor are required. Akt-mTOR pathway is known to be activated in various types of cancer, and some kinds of mTOR inhibitors are available in clinical practice. To date, however, there has been no investigation focused on Akt-mTOR pathway in pediatric INI1-deficient tumors series.

Design: We re-classified 38 pediatric INI1-deficient tumors including 26 malignant rhabdoid tumors (MRT), 4 atypical teratoid/rhabdoid tumors (AT/RT) and 8 extra-CNS unclassified sarcomas (US), according to various immunohistochemical and microRNA statuses, and analyzed the activation status of the Akt/mTOR pathway such as phosphorylated-Akt (p-Akt), p-mTOR, p-S6RP and p-4E-BP1.

Results: Histologically, these cases were re-classified into the three groups, such as "conventional type" morphologically identical to MRT, "atypical teratoid/rhabdoid (AT/R) type" morphologically identical to CNS AT/RT, and "small cell type" characterized by a proliferation of small rounded cells having scant cytoplasm without rhabdoid cells. Conventional type, AT/R type and small cell type were 26 cases (26/26 MRTs), 6 cases (4/4 AT/RTs and 2/8 USs) and 6 cases (6/8 USs), respectively. Immunohistochemical expressions for vimentin and epithelial markers were common features in conventional, AT/R and small cell type cases. In addition, microRNA analysis demonstrated that 5 conventional type cases and 1 small cell type case had very close profiles (Peason's correlation coefficient: 0.7955). Immunohistochemical positive expressions of p-Akt, p-mTOR, p-S6RP and p-4E-BP1 were observed in 20% (conventional, 15%; AT/R, 20%; small cell, 40%), 30% (conventional, 15%; AT/R, 80%; small cell, 40%), 77% (conventional, 75%; AT/R, 100%; small cell, 60%) and 37% (conventional, 25%; AT/R, 80%; small cell, 40%) of pediatric INI1-deficient tumors.

Conclusions: All extra-CNS US cases can be included to the concept of MRT, because of common immunohistochemical phenotypic expression and microRNA statuses between US and MRT. Akt-mTOR pathway was highly activated in AT/R type, compared with conventional type. Therefore, mTOR inhibitor can be a novel candidate for therapeutic target in AT/R type, and this new classification can contribute to the treatment strategy of pediatric INI1-deficient tumor.

50 Expression of Mesenchymal Developmental Antigens in Chordoma

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Background: Chordomas are thought to derive from notochordal remnants. The transcription factor brachyury is a specific marker for chordomas that is expressed in all stages of notochord formation, but expression of other developmental antigens has not been broadly studied in chordomas. This study explores the expression in chordomas of proteins important for embryonal notochord and somite development and compares it with expression in other myxoid tumors.

Design: With IRB approval, cases were retrieved from the paraffin archive, and a tissue microarray was prepared with duplicate cores from 20 chordoma cases from 12 patients, 7 cases of intramuscular myxoma, and 3 cases of extraskeletal myxoid chondrosarcoma (EMC). Immunohistochemical stains were performed for beta-catenin, Bmp2, fibronectin, Foxa2, Noggin, Notch-1, Noto, Pax8, quaking, and Sox9, and reactivity scored as positive (>10%) or weak to negative (≤10%). When discrepancies in reactivity occurred between paired cores, the higher value was used.

Results: Staining results are summarized in the table below.

Antigen	Chordoma	Intramuscular myxoma	EMC
Bmp2	19/19	4/7	2/2
Notch-1	19/19	7/7	3/3
Sox9	18/18	3/7	2/3
Noggin	17/19	7/7	3/3
Quaking	17/19	7/7	3/3
Fibronectin	15/19	0/7	0/3
Beta-catenin	13/18	0/7	0/2
Noto	7/19	0/7	2/2
Foxa2	5/19	7/7	1/2
Pax8	3/18	0/7	1/2

Over 75% of chordomas expressed fibronectin and quaking, similar to the profile of late notochord development. Other proteins important for notochord and somite development were expressed to varying degrees in all tumor types tested. Beta-catenin expression in chordoma cases differed by grade; low grade conventional tumors showed distinct membranous staining, which was absent in high grade chordomas (p=0.002). Differential staining for chordomas vs. intramuscular myxomas and EMC was identified for fibronectin (p=0.0005 vs. myxomas, p=0.02 vs. EMC). Bmp2, Sox9, beta-catenin, and Foxa2 were expressed in a significantly higher percentage of chordomas compared to myxomas (p=0.01, 0.003, 0.002, and 0.001, respectively), but not compared to EMC (p=1, 0.14, 0.11, and 0.5, respectively).

Conclusions: The expression of developmentally related mesenchymal antigens in chordomas is consistent with their origin from notochord remnants, and their expression of fibronectin and quaking is similar to cells in the later stages of notochord differentiation. Loss of beta-catenin staining correlates with transformation to high grade or de-differentiated phenotype in chordoma. Other developmental antigens are much more commonly expressed in chordoma than in other myxoid tumors.

51 Gastrointestinal Stromal Tumor Metastatic to Bone: Clinicopathological and Molecular Features of 29 Cases, Including 8 Previously Unreported Cases

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Background: Gastrointestinal stromal tumor (GIST) with metastases to bone are extremely rare, comprising less than 5% of cases, and pathologists rarely encounter such lesions in bone biopsy specimens.

Design: The available clinical, morphologic and immunohistochemical features of 29 cases of GIST with bony metastases were analyzed. 8 cases were culled from archives of 5 different institutions and 21 cases were gathered from literature review. The immunoprofile of these tumors, including KIT and DOG1 status, was investigated, and RT-PCR for KIT and PDGFRA, was performed.

Results: Male:female ratio was 18:11 with a median age of 61 years(range, 40-92 years). The most common primaries were located in stomach(24%), small intestine(24%), and extragastrointestinal sites(24%). Bone metastases were solitary in 16 cases(55%) and multiple in 13 cases(45%). Vertebrae(12), pelvis(9), ribs(9), humerus(6), femur(6), skull(4), scapula(1), clavicle(1), and mandible(1) were involved. Radiologically, all lesions were lytic with some having a sclerotic rim and adjacent soft tissue involvement. The tumors were composed of intersecting fascicles of spindle cells in 11/17(65%) cases and epithelioid cells in 2/17(12%) cases; both components were present in 4/17 cases(23%). Prominent vascular network(5/17), hyalinization(4/17), myxoid degeneration(3/17), cellular atypia(3/17), neoplastic or non-neoplastic giant cells(2/17) were encountered. In cases in which immunohistochemistry was performed, 14/17(82%) were c-kit positive, 11/14(79%) were CD34 positive, 6/8(75%) were DOG1 positive, 2/9(22%) were smooth or pan-muscle actin positive. S100(10), pancytokeratin(7), and desmin(6) were negative. KIT Exon 11 mutation was seen in 8/10 cases, one of which showed a concommitent KIT Exon 13 mutation.

Conclusions: Bone metastases from GISTs are usually seen in patients with advanced disease and are characterized by solitary or multiple lytic masses in the axial skeleton with occasional soft tissue involvement. KIT Exon 11 mutation was the most prevalent molecular alteration. Even though rare, pathologists should be aware that bony metastasis from GISTs may occur as the morphologic appearance may be quite variable.

52 R132C *IDH1* Mutations Are Found in Spindle Cell Hemangiomas and Not in Other Vascular Tumors or Malformations

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Background: Spindle cell hemangioma is a rare benign vascular tumor of the dermis and subcutaneous tissue, presenting mainly in children and young adults. The lesions can be multifocal, and are overrepresented among patients with the rare Maffucci syndrome in which patients also have multiple enchondromas. Somatic mosaic R132C hotspot mutations were recently identified in Maffucci syndrome.

Design: We evaluated the presence of *IDH* mutations in solitary and multiple spindle cell hemangiomas occurring in patients without multiple enchondromas. In addition we tested a range of other vascular lesions that enter the differential diagnosis of spindle cell hemangioma, in order to evaluate the specificity of *IDH1* mutations and to identify a possible role in the differential diagnosis of vascular lesions.

Results: In total 18 of 28 (64%) spindle cell hemangiomas demonstrated the R132C *IDH1* mutation using the hydrolysis probes assay, confirmed by Sanger sequencing. Of the 10 negative cases, 2 demonstrated a mutation in *IDH2* (R172T and R172M) by Sanger sequencing. None of 154 other vascular malformations and tumors examined contained an *IDH1* R132C mutation. R132H *IDH1* mutations were absent in all cases. All 16 spindle cell hemangiomas examined were negative for the expression of HIF-1α by immunohistochemistry.

Conclusions: In conclusion, 20 of 28 (71%) spindle cell hemangiomas harbored mutations in the *IDH1* or *-2* genes, 18 of which (90%) occurred specifically at R132C in *IDH1*. Since mutations were absent in 154 other vascular malformations and tumors, the mutation seems to be highly specific for spindle cell hemangioma within the differential diagnosis with other vascular lesions. The mutation does not induce expression of HIF-1α in spindle cell hemangioma, and therefore the exact mechanism by which mutations in *IDH* lead to vascular tumorigenesis remains to be established.

53 Massive Localized Lymphedema: A Clinicopathologic Study of 35 Patients with an Enrichment for Multiplicity

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Background: Massive localized lymphedema (MLL) is a monstrous tumefactive pseudosarcoma seen in middle aged morbidly obese adults. MLL displays a predilection for the inner thigh but other sites have been reported. Despite its initial description in 1998, the etiology remains unknown although associations with trauma, surgeries, and hypothyroidism have been reported. Herein, we report the largest study of MLL and expand upon its clinicopathologic features.

Design: Electronic medical records uncovered 35 patients with "massive localized lymphedema' from the institutional archives of The Ohio State University Wexner Medical Center between 2002 – 2012. All available H&E stained slides were reviewed.

Results: 35 patients (18 males and 17 females, age range 23-76 years, mean 49 years) presented with large soft tissue masses developing over a 5-36 months period. The majority of patients were Caucasian (n=30) with a Caucasian:African American ratio of 6:1. All patients were obese with a mean weight of 390.61 lb (range 209– 576 lb) and a mean BMI of 60.42 (range 36-81.35 kg/m^2). Endocrinopathies were present in the majority (n=18): diabetes mellitus type II (n=11), hypothyroidism (n=5), and both diseases (n=2). 2 patients had an antecedent history of trauma and 8 presented with multifocal MLL. The tissue sites included thigh (n=24), abdomen (n=12), mons pubis (n=5), scrotum (n=2), perianal (n=1) and right flank (n=1). Mostly, the clinical impression was lymphedema pseudotumor. Only 2 patients underwent MRI to rule out sarcoma. Grossly, the mean weight was 6401 grams (range 740-26940gr) and the mean size was 54 cm (range 14.8-147.6cm). Histologically, all cases showed prototypic features of MLL including variable degrees of cutaneous reactive changes, alternating edema and fibrosis of the dermis punctuated by foci of lymphangiectasia, and expansion of the fibrous septae encasing lobules of fat in the subcutis with variably atypical spindle cells.

Conclusions: We report the largest series of MLL. As MLL remains an important mimic for atypical lipomatous lesions/well-differentiated liposarcomas, it is crucial to be aware of its peculiar clinicopathologic features. Our data demonstrate this is a lesion exclusively seen in morbidly obese patients where the thigh is the most common site of involvement. We note for the first time, a marked racial predilection for Caucasians and a tendency towards multiplicity. Approximately 50% of patients in our series have an associated endocrinopathy, suggesting that these may be part of the metabolic syndrome of obesity and could play a role in its pathogenesis.

54 Are Peripheral Pure Pleomorphic Sarcomas with *MDM2* Amplification Dedifferentiated Liposarcomas?

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Background: Dedifferentiated liposarcoma (DDLPS) has been defined as a tumor composed of well-differentiated liposarcoma (WDLPS) associated with a nonlipogenic undifferentiated sarcoma and is genetically characterized by a 12q13-14 amplicon with *MDM2* amplification. Some peripheral (extremities, trunk wall, head and neck) undifferentiated pleomorphic sarcomas without areas of WDLPS present a *MDM2* amplification (PUPS-MDM2 pos).The purpose of this study is to address whether they are true DDLPS or not.

Design: The present study compared clinical data, histology, genomic profile (array-CGH) and follow-up (overall survival-OS, metastasis-free survival-MFS and local recurrence-free survival-LRFS) in PUPS-MDM2 pos and peripheral conventional (with areas of WDLPS) DDLPS (PC-DDLPS) patients retrieved from 1-the French sarcoma network RRePS (http://www.rreps.sarcomabcb.org) for systematic histological review (2010 and 2011) and 2-the Conticabase (http://www.conticabase.sarcomabcb.org)(1980 to 2011). We only considered patients with a resection of the primary tumor and with adequate sampling.

Results: We collected respectively 10 PUPS-MDM2 pos and 120 PC-DDLPS (which accounted for 25.3% of all DDLPS) in RRePS and 10 PUPS-MDM2 pos and 80 PC-DDLPS in the Conticabase. Follow–up was available for 20 PUPS-MDM2 pos and 80 PC-DDLPS. Clinical characteristics and follow-up are shown in Table. Array-CGH performed in 20 PUPS-MDM2 pos and in 20 PC-DDLPS showed a typical profile of DDLPS with a 12q13-14 amplicon.

		PUPS-MDM2 pos	PC-DDLPS	p-value
N		20	80	
Morphological features of dedifferentiated component	Spindle/pleomorphic	15	40	0.194
	Myxofibrosarcoma	3	2	
	Heterologous	2	9	
	Missing	0	29	
Age at diagnosis (years)	Median (range)	77 (49-90)	67 (18-88)	0.006
Sex	Male	11	51	0.470
	Female	9	29	
Tumor size (cm)	Median (range)	9.75 (2.5-20)	13 (2.5-50)	0.077
Tumor localization	Extremities	10	51	0.423
	Trunk wall	9	26	
	Head/neck	1	3	
Depth	Superficial	2	4	0.599
	Deep	18	74	
	Missing	0	2	
Follow-up,years	Median (95%CI)	22.9 (3.9-NA)	28.8 (15.1-40.1)	
OS rate		0.799α	0.867α	0.203β
MFS rate		0.436α	0.649α	0.146β
LRFS rate		0.697α	0.826α	0.395β

αKaplan-Meier estimate at 2 years; βLog-rank test

Conclusions: Although PUPS-MDM2 pos occur in older patients, the similarities in the histologic features, genomic profiles of PUPS-MDM2 pos and PC-DDLPS suggest that PUPS-MDM2 pos may be best positioned as part of the spectrum of DDLPS rather than as a distinct entity.

55 Common Homozygous *SMARCB1* Deletions in Both Proximal and Distal Types of Epithelioid Sarcoma (ES) – Can Be Reliably Detected by FISH in Archival Material

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Background: Although conventional and proximal types of ES show distinct anatomic presentation, morphologic appearance and clinical outcome, they are defined as a morphologic spectrum of a single pathologic entity due to their common epithelial phenotype and loss of INI-1/BAF47 expression. Despite the consistent loss of protein expression by immunohistochemistry, corresponding *SMARCB1* gene mutations/deletions have been only sporadically identified and mainly in the proximal type of ES. Our goal was to better characterize the incidence of *SMARCB1* deletions in distal/conventional-type compared to proximal-type of ES, as a proof of principle of their common pathogenesis.

Design: FISH analysis using custom BAC RP11-71G19 covering *SMARCB1* on 22q11.23 and a reference BAC probe for *EWSR1* on 22q12.2 was applied on a group of 28 ES (14 distal, 14 proximal) with classic morphologic appearance and loss of INI1 expression by IHC.

Results: Homozygous *SMARCB1* deletions were found in 13/14 distal and 12/14 proximal-type ES. An additional case with heterozygous deletion was found in a proximal-type ES. One case each showed two copies of *SMARCB1* gene, however, the lesional tissue available for analysis was quite limited. In one of the distal-type ES with homozygous *SMARCB1* deletion in the tumor, a heterozygous deletion was identified in the adjacent normal tissue in keeping with a constitutional abnormality.

Conclusions: FISH analysis using archival material detected a similarly high incidence of *SMARCB1* homozygous deletions in both distal and proximal type ES, in keeping with their common pathogenesis. The presence of a constitutional/germline *SMARCB1* deletion in one of the patients with distal-type ES further establishes a link with the atypical teratoid/rhabdoid tumor of the brain.

56 Array Comparative Genomic Hybridization Analysis of a Conventional Chordoma and an Adjacent Benign Notochordal Tumor Shows No Direct Lineage Relationship

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Background: Chordoma is a locally aggressive malignant intraosseous neoplasm with a potential for distant metastasis and with histologic features reminiscent of the embryonic notochord. Studies have revealed loss of tumor suppressors such as Rb, TSC1 and TSC2, CDKN2A/p16, CDKN2B/p15, PTEN and amplification of the transcription factor *brachyury*, often with recurring patterns of chromosomal losses (chromosome 1p, 3, 4, 9, 10, 13, and 14) and gains (chromosome 7). However, it is unclear whether these genetic alterations represent early or late changes in the pathogenic process. Benign notochordal cell tumors (BNCTs) is a well-demarcated, intramedullary, cohesive aggregates of large cells with adipocyte-like, vacuolated or eosinophilic cytoplasm. In contrast to chordoma, BNCTs lack the myxoidy extracellular matrix, have minimal

cytologic and nuclear atypia and show no evidence of invasion. Multiple recent studies have demonstrated BNCTs in close proximity to the chordomas, leading to the hypothesis that the former gives rise to the latter. However, an autopsy series show that up to 20% of the general population harbor BNCT in their vertebral bodies, opening the possibility that coexisting BNCT and chordoma may be coincidental. To this end, we have profiled a conventional chordoma and its adjacent BNCT to understand the lineage relationship between the two lesions.
Design: DNA samples were extracted from formalin fixed paraffin embedded, EDTA-decalcified chordoma and an adjacent BNCT, then hybridized to normal genomic DNA from the same patient, followed by array comparative genomic hybridization (aCGH) analysis.
Results: Our previous study has demonstrated that a BNCT lacks the complex genomic alterations commonly found in a conventional chordoma. Our current study identified heterozygous deletions of the KLF6 (tumor suppressor), HNRNPA2B1 and the MALAT1 loci, in addition to Y-chromosome loss in the BNCT - changes that are not identified in the chordoma from the same patient. Conversely, the chordoma shows commonly observed changes including the loss of chromosome 1p, 3, 4, 10, 13, and gain of chromosome 7 - none of which are present in the adjacent BNCT.
Conclusions: Our data show that the analyzed BNCT cannot be a precursor lesion of the adjacent conventional chordoma. However, the possibility of other unidentified or non-preserved BNCT giving rise to the chordoma cannot be ruled out.

57 Recurrent Amplification at 11q13.5-14.1 Targets *PAK1* as a Tumor-Promoting Oncogene in Primary Myxofibrosarcomas

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Background: Myxofibrosarcoma is genetically complex and obscure in molecular determinants of clinical aggressiveness. By using array comparative genomic hybridization to profile 12 fresh samples and 3 cell lines, one of the recurrent gained regions seen in at least 20% of samples was mapped to 11q13.5-14.1, encompassing amplified *PAK1* (p21 protein-activated kinase 1) oncogene with mRNA upregulation seen in published transcriptome (GSE21122), among others.
Design: To evaluate significance of *PAK1* gene status, mRNA abundance, and protein expression, FISH, branched-chain DNA (bDNA), and immunohistochemical assays were performed in independent myxofibrosarcoma samples, yielding 75, 79, and 98 informative cases, respectively. The results were correlated with clincopathological and tested variables, disease-specific survival (DSS), and metastasis-free survival (MFS). The oncogenic functions of *PAK1* were elucidated with **(1)** transfection of wild-type PAK1 and hyperactivating T423E mutant ($PAK1^{T423E}$) in low PAK1-expressing NMFH-2 myxofibrosarcoma cells and **(2)** short-hairpin RNA interference in PAK1-overexpressing OH931 cells.
Results: Defined as 5-fold increased signals relative to reference probe and present in 14.7% (11/75) of cases, high-level *PAK1* amplification was significantly related to higher mRNA abundance (p=0.011, bDNA assay) and immunoexpression (p=0.004), with the latter two also associated with each other and deep-seated tumors. High-level *PAK1* amplification highly correlated with higher grades (p=0.009), together with increased mRNA abundance (p=0.011) and immunoexpression (p<0.011) of PAK1. Apart from higher grades, only high-level *PAK1* amplification independently portended adverse outcomes (DSS, p=0.018, hazard ratio=3.929; MFS, hazard ratio=5.772, p=0.016). Stable PAK1-knockdown OH931 cells displayed significantly attenuated cell migration/invasion in transwell and matrigel assays and decreased cell proliferation in Brdu assay. Conversely, these aggressive phenotypes were enhanced in NMFH-2 cells transfected with wild-type PAK1 and even more drastic with $PAK1^{T423E}$ mutant.
Conclusions: High-level *PAK1* amplification contributes to mRNA and protein upregulation and promotes tumor aggressiveness via enhancing cell proliferation and migration/invasion in myxofibrosarcomas.

58 Myofibromas with Atypical Features: Expanding the Morphologic Spectrum of a Benign Entity

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Background: Typical myofibromas are biphasic tumors having a peripheral myoid cuff and a central zone of immature spindled/rounded cells. A subset of myofibromas display various atypical features that lead to a misdiagnosis of sarcoma. To more completely characterize these tumors and define their behavior we analyzed our experience with myofibromas having one or more atypical features.
Design: Out of 267 cases of myofibromas in our consultation files, 25 cases were retrieved based on reports in which atypical features were mentioned. Hematoxylin and eosin stained slides and immunostains were reviewed. Available follow-up information was obtained.
Results: The tumors presented in 15 males and 8 females [gender unknown in 2] (mean age 21 yrs; range 5 months-72 yrs) as masses of variable size (mean 2.8 cm; range 1.2 to 6.5 cm). Fourteen cases arose on the head and neck (mandible 5, tongue 2, neck 2, zygomatic bone 1, parotid 1, eyelid 1, cervical vertebra 1, scalp 1) and 11 cases on the limbs (arm 2, forearm 2, hand 3, wrist 1, foot 2, calf 1). Grossly, the lesions were tan-white to red/brown and firm. The referring or suspected diagnosis was sarcoma in 9 cases. The tumors were composed of sheets and fascicles of spindled to ovoid cells situated around a pericytomatous vasculature (19/25) with peripheral or interspersed myoid nodules (21/25) and necrosis (5/25). No tumor had significant nuclear atypia. Atypical features included hypercellular areas with a primitive appearance (20/25), infiltrating borders (19/25), intravascular growth (6/25) and perineural invasion/nerve entrapment (6/25). The mean mitotic rate was 5 mitoses/10HPF. The tumors were positive for SMA (10/10) and CD34 (2/8) and negative for other markers. Follow up in 5 patients (median 33 mos; range 2-92 mos) revealed no local recurrences or distant metastases.
Conclusions: A small subset of myofibromas display atypical features which include primitive-hypercellular areas, mitotic activity, infiltrating borders, intravascular growth and perineural invasion/entrapment and which may lead to a diagnosis of sarcoma. Based on preliminary follow up information these tumors pursue a benign course and should be included in the morphologic spectrum of classic myofibroma.

59 Epigenetic Study for Expression of MUC4 in Low Grade Fibromyxoid Sarcoma

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Background: Low grade fibromyxoid sarcoma (LGFMS) is a rare but distinctive fibromyxoid variant of fibrosarcoma, and has been shown to have characteristic chromosomal abnormalities such as t(7;16)(q33;p11), resulting in the *FUS-CREB3L2* fusion gene. Recent gene microarray and immunohistochemical analyses have shown that LGFMSs specifically expresses MUC4, a transmembranous glycoprotein. The *MUC4* gene is located on 3q29, that is not involved in the *FUS-CREB3L2* fusion gene, and the mechanism of the upregulationed *MUC4* gene expression is unclear.
Design: We examined the immunohistochemical expression of MUC4 in 24 LGFMSs, in all of which *FUS-CREB3L2* fusion had been identified, and 273 other soft tissue tumors of a wider variety than that examined in the previous studies. To evaluate the epigenetic regulation of the *MUC4*, a methylation status of the promoter CpG region of the *MUC4* gene was examined by methylation-specific PCR (MSP).
Results: Immunohistochemically, 91% (22/24) of LGFMSs and 22% (6/27) of synovial sarcomas were positively reactive to MUC4. The MUC4 expression was noted diffusely in LGFMSs, whereas focal and predominant MUC4 expression was seen in an epithelial component of synovial sarcomas. The other soft tissue tumors examined were negative. The MSP analysis revealed the promoter CpG of the *MUC4* gene was unmethylated in all the 12 LGFMSs examined including a MUC4-negative tumor, and in 11 of the 12 other soft tissue tumors including MUC4 -negative synovial sarcomas.
Conclusions: MUC4 is a sensitive and reliable immunohistochemical marker for distinguishing LGFMS from non-LGFMS tumors other than synovial sarcoma. The almost consistently but not specifically unmethylated promoter region of the MUC4 suggests an additional regulatory mechanism leading to the aberrant expression of MUC4 in LGFMSs and synovial sarcomas.

60 Pleomorphic Fibrosarcoma of Skin and Soft Tissues. Clinicopathologic, Immunohistochemical, and Ultrastructural Analysis of 145 Cases

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Background: Fibrosarcoma of skin and soft tissues as defined in the current WHO-classification represents a very rare entiy and its morphologic features may be seen in different neoplasms. We report a series of pleomorphic fibrosarcomas and discuss its relationship to other pleomorphic sarcomas.
Design: The consultation files of the authors and databases of our institutions were searched for cases coded as fibrosarcoma of pleomorphic sarcoma. All other lines of differentiation have been excluded by histological, immunohistochemical and molecular studies, and the remaining cases showing features of classical fibrosarcoma and pleomorphic sarcoma with fibroblastic/myofibroblastic differentiation were included.
Results: The 145 cases occurred in 79 men and 65 women (gender was unknown in 1 case) with a age range from 20 to 99 years (median: 69 years). The neoplasms arose on the lower (41) and upper (17) extremities, the head/neck region (29), the trunk (21), the pelvic region (7), the retroperitoneum (6), intrathoracal (3), the genital area (3), the buttock (3), the hands and feet (4), intraoral (2), the kidney (1), and in unknown location (8). 23 neoplasms arose in dermosubcutaneous location, 9 cases in the subcutis, and the other neoplasms in deep soft tissues. 18 cases showed morphologic features of classical fibrosarcoma of adults, composed of cellular fascicles of atypical spindled fibroblastic cells, in 45 cases a transition to areas of high-grade pleomorphic sarcoma was seen, and 82 cases showed features of high-grade spindle cell/pleomorphic sarcoma with fibroblastic/myofibroblastic differentiation. The mitotic rate was increased, and tumor necrosis was noted in 43 cases. Immunohistochemically, 49% of cases tested showed a focal expression of actin, 15% a focal expression of CD 34, and 78% a focal expression of CD68. The remaining antibodies were negative. FISH-analysis in selected cases did not reveal characteristic changes for neoplasms that have to be considered in the differential diagnosis. Ultrastructural analysis in 10 cases revealed features of neoplastic fibroblasts with abundant rough endoplasmic reticulum and extracellular collagen.
Conclusions: Pleomorphic fibrosarcoma represents a distinct subgroup in the spectrum of pleomorphic sarcomas of skin and soft tissues. Further clinicopathological and follow-up studies are necessary to evaluate differences to other pleomorphic sarcomas of skin and soft tissues.

61 Recurrent *NOTCH2* and *NOTCH3* Gene Rearrangements in Benign and Malignant Glomus Tumors

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Background: Glomus tumors (GT) are members of a heterogeneous family of tumors of peri-vascular modified smooth muscle cells, including among others myopericytoma, myofibroma/tosis, and angioleiomyoma. However, there are no molecular studies to date to investigate if this morphologic spectrum also shares similar genetic abnormalities.

Design: Next generation RNA sequencing was performed on one malignant visceral GT (glomangiosarcoma). Data was analyzed using FusionSeq, a modular computational tool developed to discover gene fusions. Gene fusion candidates were validated by FISH, RT-PCR and Sanger sequencing. Additional cases interrogated were 24 benign GT/glomangiomas (20 soft tissue; 4 visceral), 1 multifocal/hereditary GT, 2 visceral glomangiosarcomas, 5 myopericytomas, 7 myofibroma/toses, and 12 angioleiomyomas.
Results: A gene fusion involving *NOTCH2* and a genomic location in chromosome 5 was identified in the sequenced index case, and then validated by FISH and RT-PCR. As both *NOTCH2* and *NOTCH3* genes have similar functions in regulating vascular smooth muscle development and homeostasis, we investigated our cases for structural and numerical abnormalities in both genes by FISH. *NOTCH2* gene rearrangements were identified in 3/3 visceral glomangiosarcoma and 1/4 visceral benign GT. Rearrangements in either *NOTCH2* or *NOTCH3* were identified in 14/20 (60%) soft tissue GT, including 1/6 digital GT from one NF1 patient. Only 1/12 (8.3%) angioleiomyomas showed *NOTCH2* gene rearrangement, while all the myopericytomas and myofibroma/tosis cases were negative.
Conclusions: We describe for the first time recurrent *NOTCH2* and *NOTCH3* rearrangements in benign and malignant, visceral and soft tissue glomus tumors and in rare cases of angioleiomyoma. These findings suggest a common pathogenesis for at least some members of the perivascular myoid family of tumors. Potential use of Notch inhibitors in treatment of aggressive/malignant forms of GT has yet to be determined.

62 Suppressed Expression of Caveolin-1 Characterizes Solitary Fibrous Tumors: Comparison with Other Mesenchymal Tumors and Malignant Mesotheliomas

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Background: Solitary fibrous tumor (SFT) is a rare neoplasm, composed of bland, non-descript fibroblastic spindle cells immunoreactive to CD34. Caveolin-1 (Cav1), a scaffolding protein that regulates signal transduction and cellular metabolism, is known to play a key role in a subset of fibroblasts. For example, the cancer-associated fibroblast (CAF) is characterized by Cav1 deficiency and increased expression of pyruvate kinase isozyme M2 (PKM2). The expression status of Cav1 has not been studied in fibroblastic tumors. To clarify the nature of tumor cells in SFT and to identify diagnostic markers, we conducted immunohistochemical analysis of Cav1 and PKM2 in SFT and its mimics.
Design: 16 SFTs (5 pleural and 11 extrapleural) were immunostained with Cav1 and PKM2 antibodies. Non-SFTs that may mimic SFT were similarly examined. The latter included 16 malignant mesotheliomas, 20 fibroblastic tumors, 21 fibrohistiocytic tumors, 17 CD34-positive mesenchymal tumors, and 16 miscellaneous spindle cell tumors. Endothelium and CAFs (associated with breast cancer) were used as positive and negative controls, respectively. The staining results were scored as 0, 1, or 2.
Results: Cav1 expression was completely negative in 81% (13/16) of SFTs. In contrast, only 21% (19/90) of non-SFT tumors were negative; they included 90% (9/10) of synovial sarcoma (SS), 80% (4/5) of myxofibrosarcoma (MF), 12.5% (2/16) of malignant fibrous histiocytoma (MFH), 33.3% (2/6) of spindle cell lipoma (SL), and 40% (2/5) of dermatofibrosarcoma protuberans (DFSP). PKM2 was negative in 63% (10/16) of SFTs, whereas only 11.1% (10/90) of non-SFTs were negative for PKM2; the latter included 20% (2/10) of SS, 20% (1/5) of MF, 6.3% (1/16) of MFH, 50% (3/6) of SL, 40% (2/5) of DFSP, and 1.7% (1/6) of gastrointestinal stromal tumor. Notably, the Cav1-/PKM2- phenotype was observed in 63% (10/16) of SFTs, while only 4.4% (4/90) of non-SFTs exhibited this phenotype.
Conclusions: Suppressed expression of Cav1 characterizes fibroblastic tumor cells of SFT, and this finding may be useful for diagnosis. Furthermore, the Cav1-/PKM2- phenotype is highly characteristic of SFT in contrast to CAF, SS, and MF, which are mostly of the Cav1-/PKM2+ phenotype. Cav1 deficiency may be related to the distinct hemangiopericytomatous vascular proliferation that is commonly observed in SFT and SS.

63 Dedifferentiated Liposarcoma Is the Most Frequent Liposarcoma Subtype. A Study from the French Sarcoma Group on the Subtype Frequencies in a Series of 1384 Liposarcomas with a Systematic Molecular Characterisation

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Background: The standards for subtyping liposarcomas (LPS), and particularly for identifying dedifferentiated LPS, have recently changed with the more and more frequently use of molecular analysis. Therefore, the exact frequency of LPS subtypes is unknown.
Design: A national network of pathologists has been set up in France for a systematic review of every new sarcoma. Moreover, a molecular test is systematically performed for every suspicion of translocation (FISH or RT-PCR), *MDM2* amplification (FISH, Q-PCR or array-CGH) or specific mutation. The following data are collected in a shared database (http://www.rreps.sarcomabcb.org): sex, age, location, depth and size of tumor, date and type of sampling, type and result of molecular test and histotype.
Results: From January 1st 2010 to June 30th 2012, 5665 soft tissue sarcomas were collected with 1388 LPS (24%) with 562 (40%) dedifferentiated LPS (DD-LPS), 545 (39%) atypical lipomatous tumors/well differentiated LPS (ALT/WD-LPS), 205 (15%) myxoid/round cell LPS (MR-LPS), 62 (5%) pleomorphic LPS (P-LPS) and 14 (1%) NOS-LPS. In the category of ALT/WD, DD, and MR-LPS, molecular analysis was positive in 1222 cases (93%), negative in 3 cases (MR-LPS) non contributive in 35 cases (2.7%) and not done in 52 cases (4%). Sex, age and location according to histotype is shown in the Table 1

Table 1: Sex, age and location according to LPS subtypes

Type of LPS	ALT/WD-LPS	DD-LPD	MR-LPS	P-LPS
Sex (M/F)	1.15	1.8	1.5	2.1
Median age (years)	65	70	46	65
Limbs (n*)	253	72	167	30
Internal trunk (RP**)	170 (120)	425 (312)	9 (0)	11 (7)
Trunk wall	108	52	29	20
Head and Neck	14	13	0	1

n*: number of cases; RP**: retroperitoneum

Conclusions: In this prospective systematic review of sarcomas, DD-LPS and ALT/WD-LPS represent almost 80% whereas MR-LPS represent only 15% of all LPS. With a more systematic molecular analysis, DD-LPS is actually more frequent than previously thought.

64 HEY1-NCOA2 Fusion Transcript Detection Is a Valuable Diagnostic Adjunct in the Diagnosis of Mesenchymal Chondrosarcoma

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Background: Mesenchymal chondrosarcoma (MC) is a rare sarcoma of children and young adults arising in bone and soft tissues. Morphological diagnosis is often challenging since its salient biphasic morphology is often not represented on small biopsy material. Appearances also overlap with other entities. Novel Hey1-NCOA2 fusion transcripts were recently identified in MC suggestive that this is the defining genetic alteration which can be exploited for diagnostic use.
Design: RNA was extracted from 12 MC from 12 patients (7 biopsies, 5 resection specimens). RNA quality was assessed by beta-actin amplification (98 bp amplification product). HEY1-NCOA2 fusion transcript were detected by RT-PCR (119 bp amplification product) and confirmed by sequencing.
Results: Adequate RNA could be extracted from 9 out of 12 MC. HEY-NCOA2 fusion transcripts were detected in all 9 cases. Sequencing confirmed the exact in-frame HEY1 exon4–NCOA2 exon13 fusion as described in the initial cases. In two positive biopsies a tentative diagnosis of MC was given based on limited material highlighting the diagnostic benefit of transcript detection.
Conclusions: HEY1-NCOA2 fusion transcript is a consistent finding in MC. Detection by RT-PCR is a useful diagnostic adjunct especially in limited biopsy material. In contrast to other translocation sarcomas the fusion transcript is identical requiring only a single primer pair.

65 Clinicopathological Spectrum of 14 Myoepithelial Tumors, Primarily Involving Soft Tissues, Including Immunohistochemical Profile and EWSR1 Rearrangement in a Subset of Cases

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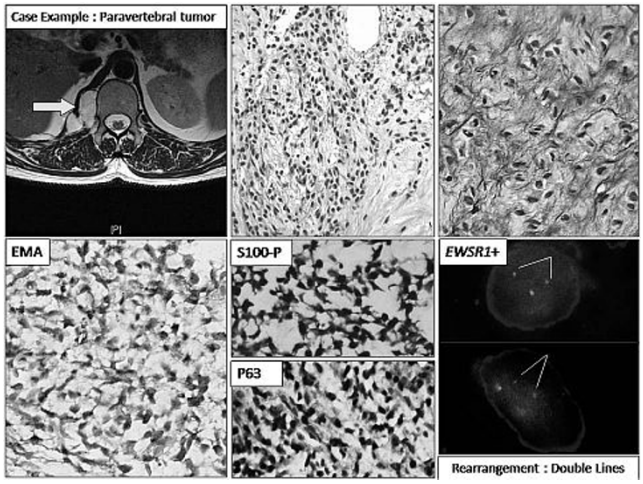
Background: Primary soft tissue myoepithelial tumors (METs) are rare. Recent studies have shown *EWSR1* rearrangement in certain METs.
Design: Herein, clinicopathological features of 14 primary soft tissue METs, included after critical review, are presented with *EWSR1* results.
Results: Fourteen tumors occurred in 12 men and 2 women within age-range of 18-60 years (mean 39.2) in upper extremities (4)(28.5%), chest wall 3(21.4%), paravertebral region 3(21.4%), pelvis 2(14.2%) and lower extremities (2)(14.2%). Tumor size varied from 2-21.6 cm (mean 8.7). Microscopically, most tumors were at least focally circumscribed, including 5 benign and 9 malignant types. Various patterns, cells and matrix-types were noted, commonest being cord-like and diffuse arrangement of polygonal cells in a myxoid matrix, un-associated with duct formations. By IHC, tumors were positive for EMA (10/12)(83.3%), CK/MNF116(3/12)(25%), p63(7/10)(70%), CD10(4/6)(66.6%), calponin (6/6)(100%), S-100P(11/13)(84.6%), GFAP (6/12)(50%), SMA(3/9)(33.3%), vimentin(4/4)(100%), INI1/SMARCB1(6/10)(60%), brachyury(0/11) and CD34(0/5), including 92.8% positivity for an epithelial marker. *EWSR1* gene rearrangement was detected in 3/6 (50%) METs (1 benign, 2 malignant); in an eccrine porocarcinoma (AE1/AE3+,EMA+,CEA+,p63+,CK5/6+) and was absent in a myxoid-type epithelioid sarcoma (CK+,EMA+,CD34+,vimentin+) that formed close differentials.



All 11 patients with known treatment details underwent tumor resection. Three tumors (2 malignant, 1 benign) resected with unknown marginal status recurred; 2 patients died and a single patient with myoepithelial carcinoma, who underwent wide-excision, is disease-free.

Conclusions: This study testifies a wide spectrum of soft tissue METs, including benign and malignant subtypes that are best treated with wide-excision. EMA and S-100P are optimal markers in our settings that should be supplemented with AE1/AE3, p63, GFAP and calponin in certain cases. Morphological features must be correlated with IHC results. Brachyury is useful in separating parachordoma/myoepithelioma from chordoma. Certain METs are INI1-deficient. *EWSR1* rearrangement mostly occurs in deep-seated METs, irrespective of benign or malignant types.

66 The Role of HER3 (erbB3) Overexpression in Peripheral Nerve Sheath Tumors (PNST) as a New Therapeutic Target

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Background: Malignant peripheral nerve sheath tumors (MPNST) comprise 5-10% of sarcomas. Prognosis is poor and the search for new treatments is ongoing. HER3 is a crucial receptor for neuregulin signalling in Schwann cells but to date, has not been studied in sarcomas. Our aims were to determine the prevalence of HER3 receptor expression in soft tissue tumors, including MPNST, and to evaluate the impact of HER3 inhibition in a subgroup of mesenchymal tumor cell lines.

Design: HER3 expression was evaluated by immunohistochemistry in a total of 50 benign and 71 malignant mesenchymal tumors, including 16 neurofibromas, 16 schwanomas, 14 MPNST and 75 other lesion (uterine and non-uterine leiomiomas and leiomiosarcomas, synovial sarcomas, undifferentiated pleomorphic sarcomas (UPS)). HER3 expression was also evaluated by western blot in 5 different mesenchymal cell lines including RT4 (murine schwann cells) and NF1 (malignant peripheral nerve sheath tumor in NF1 syndrome). Finally, HER3 expression was inhibited by the infection with specific short hairpin RNA (shRNA) in all of them. All the experiments were done by duplicate.

Results: HER3 overexpression was found in 31% of cases. Significantly, HER3 positivity was present in 50% of PNST, 75% of schwanomas, 50% of MPNST and 25% of neurofibromas. In contrast, HER3 positivity was observed in 20% of other mesenchymal tumors. Moreover, HER3 expression was found to be overexpressed in 4 of the 5 cell lines studied and their stable knockdown of HER3 expression induced a decreased of the proliferation rate in all of them and a marked lost of cell viability in the NF1 derived malignant cells.

Conclusions: HER3 overexpression is frequently found in PNST, including 50% of MPNST. Moreover, the *in vitro* knockdown of HER3 expression clearly has an impact on proliferation rates and viability of mesenchimal tumor cells. These results support the rationale of developing new therapeutic approaches in MPNST based on agents which can block Her3 signaling pathway.

67 Gastrointestinal Stromal Tumors (GISTs): Molecular Analysis of *KIT/PDGFRA/BRAF* Genes Refines Risk Assessment (Register Study)

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Background: We analyzed a subset of naive GISTs from the REGISTER series, to assess whether *KIT/PDGFRA/BRAF* mutations are relevant to prognosis.

Design: DNA was analyzed in 451/526 GISTs previously evaluated to generate a OS nomogram, based on age, tumor site, as well as mitotic index (MI) and size both considered as continuous variables. Univariable and multivariable analyses were performed. A backward selection procedure was applied to the multivariable model to automatically generate prognostically homogeneous groups.

Results:

Gene	Exon (ex)	Cases(%)
KIT	9	32(7,1)
KIT	11	253(56,1)
KIT	13	4(0,9)
KIT	17	3(0,6)
PDGFRA	12	10(2,2)
PDGFRA	14	7(1,6)
PDGFRA	18	78(17,3)
BRAF	15	5(1,1)
WT cases	-	59(13,1)

KIT ex 11 mutations included 21 557-558 deletions. *PDGFRA* ex 18 mutations comprised 60 Asp842Val substitutions. Median MI was more than 2,6-fold and 4-fold higher in 557-558 del group compared to the all series and Asp842Val group, respectively (8 vs 3 and 2 mitoses/50HPF, respectively, p=0,02). Proportion of 557-558 del cases was higher in colorectal GISTs (16%) compared to other sites (5,9% in stomach and 0 in small intestine). The poorest OS at 120 months was observed among *KIT*-mutated patients (46.3%), whereas *PDGFRA*-mutated and WT patients had similar OS (67.5% and 62.5%, respectively). In the final model all the nomogram variables retained their independent value. The molecular variable defined 3 prognostic groups (p=0.001) with improvement of model discriminative ability as compared with nomogram variables only (C=0.732 vs 0.725). The most indolent group consisted of *PDGFRA* ex 12, *BRAF* and *KIT* ex 13 mutated cases. The intermediate group included WT cases, *KIT* ex 17 and *PDGFRA* ex 14-mutated cases as well as Asp842Val cases (group 2 vs group 1 HR 3.06, CI 1.09, 8.58). The most aggressive group comprised *KIT* ex 9 and 11 as well as *PDGFRA* ex 18 mutated cases except for Asp842Val cases (group 3 vs group 1 HR 4.52, CI 1.65, 12.37). There was no difference in outcome between del557-558 and other ex 11 mutations, a datum probably related to 557-558 del association with colorectal site and high MI.

Conclusions: We identified 3 molecular groups with distinct prognosis, refining risk assessment.

68 A Contemporary Reassessment of Myxofibrosarcoma (MFS)

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Background: Myxofibrosarcoma (MFS), also known as myxoid MFH, is a relatively common soft tissue sarcoma that typically involves the extremities of older adults. Since its original description, diagnostic criteria have varied. We have noted that deep-seated undifferentiated sarcomas with variable myxoid stroma have been increasingly misclassified as myxofibrosarcoma. Different tumor characteristics have been variably associated with clinical outcome; some grading systems seem to be more powerful predictors of prognosis than others. Recent genetic studies suggest that MFS may be characterized by loss of *RB1*.

Design: We conducted a single institution database search for MFS cases. After excluding recurrences and reclassifying cases that did not meet diagnostic criteria, the clinicopathological characteristics of 32 primary myxofibrosarcomas were reviewed and statistical analysis performed to identify factors predisposing to local recurrence and/or metastasis. *RB1* immunohistochemistry was performed in 13 cases.

Results: The cohort included 18 (56%) females and 14 (44%) males with a mean age of 67 years (range 38-95 years). The most common location was the thigh (31%, n=10) followed by the arm (19%, n=6). Fourteen tumors (44%) were superficial, 12 (37%) deep seated and 6 (19%) had both superficial and deep components. Mean follow up was 35 months (range 23 days to 9.7 years). Nine (28%) were lost to follow up. Eight (33%) developed 1 or more recurrences and six (26%) developed metastases, with the most common site being the lung. None of the variables studied reached statistical significance with univariate analysis using Cox proportional hazard models predicting local recurrence and/or metastasis (p-values of 0.70 for depth, 0.08 for a positive margin, 0.45 for FNCLCC grade, 0.19 for size and 0.72 for percentage of myxoid stroma/cellularity). *RB1* immunoreactivity was lost in 1 of 13 cases; 1 case showed a heterogeneous staining pattern.

Conclusions: In this limited study, the results suggest that positive margin status (p = 0.08) may be the most powerful predictor of recurrence and/or metastasis in myxofibrosarcoma. *RB1* is lost in only a small percentage of cases. Thus, the molecular pathogenesis of most cases of MFS remains to be determined.

69 A Subset of Gastrointestinal Stromal Tumors Previously Regarded as Wildtype GISTs Carries Sporadic Activating Mutations in the Extracellular Ligand Binding Domain of *KIT* (Exon 8, p.D419del)

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Background: About 15% of GISTs carry wild type sequences in all hot spots of *KIT* and *PDGFRA* (wt-GISTs). These tumors are currently defined by having wild type sequences in exons 9, 11, 13, and 17 of the *KIT* gene and exons 12, 14, and 18 of *PDGFRA*. Until now, the analysis of further exons is not recommended. However, we have previously published a report on a *KIT* exon 8 germline mutation which was associated with familial GISTs. Thus, we aimed at investigating whether *KIT* exon 8 mutations might also occur in sporadic GISTs.

Design: We therefore screened a cohort of 145 wt-GISTs for somatic mutations in *KIT* exon 8. All tumors were immunohistochemically positive for KIT and DOG-1.

Results: Two primary GISTs with an identical exon 8 mutation (p.D419del) were detected, representing 1.4% of all cases investigated. Based on 1338 GISTs from our registry the overall frequency of *KIT* exon 8 mutations was 0.15%. The first case was found in a 53-year-old male patient. The tumor showed a biphasic spindled-epithelioid pattern with high proliferative activity (13/50HPF). Furthermore, a second low proliferative spindle cell pattern was noted (4/50 HPF). The patient suffered from

multiple peritoneal metastases 29 months later. In case 2, the 57-year old female patient followed, however, a benign clinical course under adjuvant treatment. 19 month after removal of the GIST, there is no evidence of recurrence. Skin changes or clinical signs of mastocytosis were not noted in both patients.
Conclusions: We report here for the first time on sporadic mutations in the extracellular ligand binding domain (exon 8) of *KIT*. The same type of mutation was previously reported in familial GISTs. In this report we could identify a kindred with both GISTs and mastocytosis. We could show, that the mutation results in constitutively activated *KIT*, which can be successfully inhibited by imatinib. We conclude that a small proportion (about 1%) of GISTs being formerly reported as "wild type" might in fact be *KIT* exon 8 mutated. Given that (1) this subtype was shown to be activating and imatinib sensitive *in vitro* and (2) our follow up data, suggesting a favourable outcome for imatinib treated patients, we suppose that the screening for *KIT* exon 8 mutations should be included in the diagnostic work up of GISTs and that patients with exon 8 mutation and significant risk for tumor progression should be treated with imatinib.

70 Histological Analysis of Vascular Involvement Suggests an Invasion-Independent Metastatic Mechanism in Alveolar Soft Part Sarcoma

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Background: Alveolar soft part sarcoma (ASPS) is a rare soft tissue tumor characterized by pseudoalveolar growth of polygonal cells associated with abundant sinusoidal vessels. ASPS has a high proclivity to blood-borne metastasis and is resistant to standard chemotherapy. Recently, an invasion-independent mechanism of metastasis has been postulated for a subset of human carcinomas (Am J Pathol. 2002;160(6):1973), in which cancer cells are shed into vessels, whilst being completely enveloped by endothelial cells, and are subsequently entrapped at recipient organs. This contrasts with the canonical pathway of metastasis, in which cancer cells actively break through vascular walls without being enveloped by endothelial cells. A previous study (BMC Med. 2004;2:9) showed that invasion-independent metastasis tends to occur in carcinomas with sinusoidal vessels. Because ASPS harbors abundant sinusoidal vessels, we hypothesized that the invasion-independent metastatic mechanism may play a role in this disease. To test this, we examined ASPS surgical specimens, paying particular attention to the mode of vascular involvement.
Design: A total of 32 ASPSs surgically resected from 27 patients were retrieved. All the H&E-stained slides were reviewed, and the detailed morphology of vascular involvement was analyzed. Vascular involvement was defined as tumor cells lying completely within vascular spaces. For objective evaluation, we limited analysis to vascular involvement located outside the contour of tumor mass; intratumoral events were excluded from analysis. All vascular involvements were immunostained for CD31.
Results: A total of 191 instances of unequivocal vascular involvement were identified in 84 slides from 20 ASPSs. In almost all (185/191, 97%) of the observed instances of vascular involvement, the intravascular tumor cells were in the form of variously sized cohesive clusters that were tightly enveloped by endothelial cells. Complete endothelial envelopment was confirmed by CD31 immunostaining. Dyscohesive intravascular tumor cells without endothelial wrapping were extremely rare.
Conclusions: Our findings suggest that, in ASPS, the canonical invasive pathway is not followed for vascular involvement, but instead, the invasion-independent mechanism is employed for entry into circulation. Because the latter mechanism is reportedly dependent on tumor angiogenesis and vascular remodeling, our data provide a morphological rationale for the use of anti-angiogenic therapy to treat ASPS.

71 Primary Myoepithelioma of the Orbit

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Background: Myoepithelioma is an uncommon tumor that has been described in a variety of different anatomic sites including the soft tissues. Histologically, its morphologic heterogeneity can cause confusion with a variety of different neoplasms. Myoepithelial tumors arising in the orbit are rare and to increase our understanding of these neoplasms we reviewed our experience with 3 cases.
Design: The study cohort was identified from one of the author's consultation files and from the surgical pathology files of Massachusetts General Hospital. The clinicopathologic characteristics of the tumors including their histologic and immunohistochemical features, and status of translocation involving the *EWSR1* gene were analyzed. Ultrastructural study was also performed in 1 case.
Results: The patients included 3 females ages 5, 13 and 16. The tumors arose in the orbital soft tissues. Histologically, the tumors were solid, had a lobulated growth pattern and were composed of spindle to epithelioid cells with eosinophilic cytoplasm. The tumor stroma varied from myxoid to hyalinized. In one case scattered duct-like structures lined by cuboidal cells were present. The tumor cells lacked cytologic atypia, mitotic activity and necrosis. Immunohistochemically, the tumor cells expressed s-100, EMA, keratin, p63, desmin, SMA and calponin. The tumor cell were negative for GFAP, myogenin, MYOD-1 and CD34. Electron microscopy performed on Case 3 showed tumor cells with reduplicated basal lamina, short cellular processes and cytoplasmic filaments with focal densities, features consistent with myoepithelial differentiation. Fluorescent in situ hybridization performed on Cases 2 and 3 for translocation involving the *EWSR1* gene on chromosome 22 was positive for a rearrangement. The tumors were treated with conservative resection and follow-up ranged from 3 to 46 months. Case 3 had a local recurrence 18 months after initial resection and underwent subsequent resection, and currently has no evidence of disease based on a recent MRI.
Conclusions: Myoepithelial tumors of the orbit are rare and can be confused with a variety of different neoplasms including rhabdomyosarcoma. Immunohistochemically, they often express S100, EMA, keratin and can have rearrangement of the *EWSR1* gene. Biologically, these tumors can locally recur and the goal of treatment, if possible, is surgical resection with negative margins.

72 Correlation of MDM2, p53 and MIB-1 Expression with Response to Neoadjuvant Chemotherapy in High-Grade Extremity Osteosarcomas and Clinical Outcome

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Background: There is a need for identifying predictive markers in high-grade osteosarcomas which would help predict response to neoadjuvant chemotherapy (NACT), so that the treatment can be individualised. This study was carried out to correlate immunohistochemical expression of MDM2 (murine double minute 2), p53 and MIB-1 in pre-treatment biopsies of high-grade extremity osteosarcomas with percentage of tumor necrosis (chemotherapy response) on resected specimens and also with clinical outcome, with an objective to find predictors of NACT response.
Design: Immunohistochemistry for MDM2, p53 and MIB-1 was performed on 100 pre-treatment biopsies of high-grade extremity osteosarcomas. MDM2 and p53 of ≥10% and MIB-1 of >10% tumor cell nuclear staining were considered as positive staining. Chemotherapy response was graded according to Huvos grading system, wherein good responders (GR) had >90% tumor necrosis and poor responders (PR) had ≤90% tumor necrosis. P values were calculated by Log Rank test, using SPSS version 16 ($P<0.05$ was significant) and survival outcomes by Kaplan-Meier curves.
Results: The mean Disease Free Survival (DFS) for GR (47 months) was significantly higher than for PR (28 months) (P=0.02). Similarly, mean Overall Survival (OAS) for GR (61 months) was significantly higher than for PR (36 months) (P=0.03). More GR (54.3%) had MDM2 negative tumors than PR (33.3%) (P=0.05). However the mean DFS for MDM2 negative tumors was 41 months in contrast to 42 for MDM2 positive cases (P=0.7). Mean OAS for MDM2 negative cases was 49 months in contrast to 64 for MDM2 positive cases (P=0.37). There was no significant correlation between overexpression of p53 and MIB-1 with NACT response. Mean DFS for p53 positive cases was 42 months and for p53 negative cases was 45 (P=0.97). Mean OAS for p53 positive cases was 54 months and for p53 negative cases was 61 (P=0.8). There was no significant correlation between high MIB-1 expression and DFS (P=0.89). Mean OAS for cases with high MIB-1 was 52 months and with low MIB-1 was 58 (P=0.73).
Conclusions: This study reinforces significant association of grade of tumor necrosis in NACT treated high-grade osteosarcoma cases and clinical outcome. However within the studied markers, although MDM2 negativity significantly correlated with good NACT response, p53 negativity correlated with improved survival and MIB-1 over expression came forth as a marker of biologic aggressiveness, none of these markers revealed any statistically significant results vis a vis clinical outcome implying lack of role as predictive markers.

73 Prognostic Features of Myxofibrosarcoma: A Clinicopathologic Study Assessing the Impact of FNCLCC Grading System

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Background: There has been variability in the diagnostic and grading criteria of myxofibrosarcoma. To address these issues we undertook a retrospective review of myxofibrosarcomas to assess the prognostic effectiveness of the widely accepted FNCLLC grading system and to determine what clinicopathologic features are important in prognosis.
Design: The Mayo Clinic pathology archive was searched for primary, non-treated cases of myxofibrosarcoma. 32 cases of myxofibrosarcoma meeting these criteria were evaluated with regards to size, atypia (mild, moderate, severe), % myxoid area (<5%, 5-50%, >50%), mitotic rate (per 10 high power fields), and % tumor necrosis (0, ≤50%, >50%). All cases were also graded using the FNCLCC grading system.
Results: Table 1 summaries the histopathologic features of 32 studied cases. The tumors occurred in 19 females and 13 males, ranging from 27.8 to 87.7 years (median 69.4 years). The lesions ranged in size from 1.5 to 16.5 cm (mean 6.2 cm) and involved the lower (19) and upper (7) extremities, trunk (2), head (2), shoulder (1), and buttock (1). FNCLCC grades were: Grade 1 (12), Grade 2 (14), and Grade 3 (6). Follow-up information was available for 31 patients (range 0.1 to 17.9 years; mean 4.2 years; median 3.0 years). The 5-year overall survival and recurrence/metastasis-free rates were 70.9 % and 59.6%, respectively. By Cox univariate analysis, tumor size greater than 5 cm (p=0.0004) and presence of tumor necrosis (p=0.0262) correlated with increased mortality. Other morphologic parameters, including FNCLCC grade (p=0.14), degree of atypia (p=0.82), mitotic count (p=0.47) and myxoid component (p=0.84), showed no significant association with outcome.
Conclusions: The tumor size (>5 cm) and presence of tumor necrosis were identified as statistically significant factors for poor outcome. This data also suggests that the current FNCLCC grading system may not be useful as a prognostic indicator for myxofibrosarcoma. The percentage of myxoid area did not correlate with outcome, suggesting that tumors with any myxoid component (even <5%) be considered in the spectrum of myxofibrosarcoma.

Histopathologic findings of 32 myxofibrosarcomas

	Total (N=32)
% Myxoid	
<5%	7 (21.9%)
5-50%	8 (25%)
>50%	17 (53.1%)
Cytologic atypia	
Mild	1 (3.1%)
Moderate	12 (37.5%)
Severe	19 (59.4%)
Mitotic rate	
<10HPF	19 (59.4%)
10-20HPF	5 (15.6%)
>20HPF	8 (25%)
% tumor necrosis	
0	18 (56.3%)
≤50%	11 (34.4%)
>50%	3 (9.4%)
FNCLCC Grade	
1	12 (37.5%)
2	14 (43.8%)
3	6 (18.8%)

74 Myxochondroid Fibrous Pseudotumor: A Distinctive, Previously Undescribed Pseudoneoplasm of the Foot with a Predilection for Adolescent Males

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Background: Cartilaginous tumors of soft tissue are uncommon, with benign chondromas of soft parts greatly outnumbering rare soft tissue chondrosarcomas. Over the past several years we have seen in consultation a distinctive, benign-appearing chondroid soft tissue tumor of the foot, that differs in a number of respects from chondroma of soft parts. Herein we report our experience with this distinctive lesion.

Design: A retrospective review of all cases from the foot in our soft tissue consultation archives identified 6 similar cases, most often previously coded as "fibroconnective tissue with chondroid metaplasia". All cases were submitted in consultation due to concern for a neoplastic process, in particular chondroma of soft parts or fibroosseous pseudotumor of the digits. Clinical information, including patient follow-up, was obtained from the referring pathologists and clinicians.

Results: The patients were 4 adolescent males (ages 11-18 years, mean 14 years) and 2 older women (ages 49 and 65 years). All cases occurred in the soft tissue of the feet, including 4 cases confined to the toes, and presented as non-specific, variably painful masses. Radiographic studies, available in 3 cases, did not show bone involvement. The lesions were characterized by a variably cellular proliferation of bland fibroblastic cells in a fibromyxoid background, and in areas showed distinct basophilia and a chondroid appearance. Intralesional cystic change and a variety of reactive changes in the surrounding connective tissue were present. Features of chondroma of soft parts, such as true cartilaginous foci with lacunae, flocculent calcification, and giant cells, or of fibrosseous pseudotumors of the digits, such as woven bone production, were absent. Clinical follow-up (6 patients, 2-67 months, mean 27.6 months) showed all patients to be without recurrent disease.

Conclusions: We have identified a morphologically distinctive lesion of the foot, most often occurring in adolescent males, termed "myxochondroid fibrous pseudotumor". The clinical and histopathological features of this lesion strongly suggest that it is a reactive, pseudoneoplastic process, rather than a true neoplasm. Awareness of the unique features of this lesion should allow its ready distinction from chondroma of soft parts, fibroosseous pseudotumor of the digits, and other cartilage-forming lesions of the foot, such as calcifying aponeurotic fibroma.

75 Loss of INI1 Expression in Extraskeletal Myxoid Chondrosarcoma

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Background: Extraskeletal myxoid chondrosarcomas (ESMC) are rare sarcomas of uncertain differentiation that often occur in the deep soft tissue of the lower extremity (LE) with metastases often detected during long-term follow up. Previously, INI1 loss was reported in a subset of ESMC. We investigated SMARCB1/INI1 expression by immunohistochemistry and examined for its prognostic significance.

Design: 18 cases of ESMC were retrieved. IHC was performed following antigen retrieval using a mouse anti-SMARCB1/INI1 antibody (1:50; BAF47; BD Biosciences). INI1 immunoreactivity was graded for extent (0, no staining; 1+, <10%; 2+, 10% to 25%; 3+, 26% to 50%; 4+, 51% to 89%; and 5+, 90% to 100%) and intensity (weak, moderate, strong). 0 to 3+ staining were considered to be loss of INI1 expression. All 18 cases were assessed by LSI *EWSR1* dual-color, break-apart probe (Abbott Molecular/Vysis, Des Plaines, IL).

Results: Demographics were as follows: M:F ratio of 14:4 with median age of 54 yrs (range:29-79). ESMC cases involved the LE (9/18, 50%), arm and buttock (2/18 each, 11%), trunk (2/18, 11%), and metastatic sites (3/18). *EWSR1* rearrangement was detected in 12/15 (80%) cases. 9 cases showed diffuse (5+ and 4+) strong labeling for INI1 while variable loss of INI1 expression was seen in 9/18 (50%) cases: 3 with or 3+ weak/moderate labeling, 3 with 1+ or 2+ weak labeling, and 3 with complete loss of expression. Clinical follow-up (F/U) was available in 17/18 pts (median: 83 mos; range: 38-274 mos). In pts whose tumors showed signficant INI1 loss (0-3+), 7/9 (78%) had metastatic disease (4 with had multiple); 3/9 (33%) were DOD with median overall survival (OS) of 57 mos (range: 41-79) and median disease-free survival (DFS) of 31 mos (range: 16-37); 6/9 (67%) were AWD with median F/U of 76 mos (range: 38-274) and median DFS of 39 mos (range: 7-227). Of the pts whose tumors showed complete INI1 retention, 7/8 (87.5%) were AWD with median F/U of 116 mos (range: 49-262) and median DFS of 57 mos (range: 1-243); 1/8 (12.5%) DOD (OS: 83 mos; DFS: 26 mos).

Conclusions: In this study, 50% of ESMC cases showed variable loss of INI1 expression. Patients whose tumors had loss of INI1 had lower OS and DFS. INI1 loss in ESMC may play a role in disease progression and may serve as a potential prognostic marker.

76 ERG and FLI1, an Ets Family Transcription Factor and Vascular Marker, Expression in Epithelioid Sarcoma: A Potential Diagnostic Pitfall

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Background: Epithelioid sarcoma (ES) is a rare, aggressive keratin-positive sarcoma that co-expresses CD34 in 50% of cases and may show cystic degeneration/hemorrhage, mimicking an epithelioid angiosarcoma. Recently, a case of ES was noted to label for ERG, an ETS family regulatory transcription factor that is associated with endothelial differentiation and is a reliable marker for vascular tumors. We investigated the prevalence of nuclear expression of ERG and FLI1, a homologous transcription factor, in ES.

Design: A formalin-fixed paraffin-embedded tissue microarray of 37 ES was examined. Immunohistochemistry was performed following antigen retrieval using a mouse anti-ERG monoclonal antibody to the N-terminus (1:30; 9FY; BioCare Medical), a rabbit anti-ERG monoclonal antibody to the C-terminus (1:2000; EPR3864(2); Epitomics) and a mouse anti-FLI1 monoclonal antibody (1:100; G146-222; BD Biosciences). The extent of immunoreactivity was graded according to the percentage of positive tumor cell nuclei (0, no staining; 1+, <5%; 2+, 5% to 25%; 3+, 26% to 50%; 4+, 51% to 75%; and 5+, 76% to 100%), and the intensity of staining was graded as weak, moderate, or strong.

Results: Nuclear staining for the N-terminus of ERG was seen in 19/28 cases: 10 with diffuse (4-5+) strong/moderate labeling; 1 with 2+ moderate labeling and 8 with weak labeling (1-4+, 2 each). Focal staining for the C-terminus of ERG was seen in only 1/29 case (1+ moderate). FLI labeling was seen in nearly all (28/30) cases: 16 with diffuse (5+) predominantly moderate labeling, and 8 cases with diffuse (5+) weak labeling. The remainder had variable moderate (1-3+) or weak (1-4+) FLI1 staining. CD34 was positive in 8/16 cases.

Conclusions: ES can express both ERG and FLI1, which may be a source of diagnostic confusion for a vascular tumor. ERG antibody selection is critical, as those directed against the C-terminus is much more specific for endothelial differentiation than those against the N-terminus. ERG may be of value in the differential diagnosis of epithelioid sarcoma, but should be used in the context of a panel of other antibodies including cytokeratins and INI1.

77 Metastatic Melanoma to Bone: A Clinicopathological Study of 236 Cases

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Background: Metastatic melanoma to bone is uncommon. It can be a source of diagnostic confusion in the differential of poorly differentiated malignant bone tumors. The 10-year experience of metastatic melanoma involving bone at a major cancer center was examined.

Design: The pathology files were reviewed from 2002-2012 for metastatic melanoma involving bone in center where over 9060 patients with primary melanomas was seen. Cases of direct extension originating in the skin and soft tissue were excluded. Clinical and radiological studies were reviewed.

Results: Two hundred thirty six cases were identified. The median age was 53 (range, 24 to 83) yrs with a male to female ratio of 141:95. Sites of involvement include the vertebrae 27%(64/236), pelvis 21%(49/236), femur 17%(40/236), sacrum 14%(34/236), humerus 9%(20/236), rib 5%(11/236), fibula 4%(10/236), and skull 3%(8/236). In 97%(229/236) of cases, patients had multiple metastasis and 89%(194/217) were radiographic lytic lesions. The mean time from primary tumor diagnosis to bone metastasis was 148(range, 0-320)mo. Location of primary melanomas included head and neck 29%(68/236), back 25%(59/236), shoulder 16%(38/236), mucosal 5%(12/236), thigh 4%(10/236), upper extremity 4%(10/236), abdomen 2%(5/236), elbow 0.4%(1/236), and unknown 14%(33/236). Primary melanoma types included nodular melanoma 54%(128/236), superficial spreading 31%(74/236), lentigo maligna 7%(18/236) acral lentiginous 3.4% (8/236), and mucosal 3.4% (8/236). Clinical outcome was available in 230/236 patients(median, 49 mo, range:1-1364 mo): 148/230 (64%) died of disease, 82/230 (36%) were alive with disease and six patients were lost to follow up. The median time to death after diagnosis was 81 (range:7-1364) mo, after first metastasis 28 (range:1-134)mo, and after first bone metastasis was 12(range:1-40) mo. Significant differences in outcome survival were identified based on general metastatic location (best to worst OS, long bones, vertebrae, pelvis, thoracic, skull, $p<0.001$). No significant difference was observed among primary melanoma subtypes ($p=0.124$).

Conclusions: This is the largest series of metastatic melanoma to bone. Metastatic melanoma to the bone is uncommon (3%) and can be the first site of metastases for patients. Bone metastases herald a poor prognosis and site may be prognostically significant. Nodular melanoma was the most common histologic subtype with the back being the most common location of primary origin.

78 MDM2 and CDK4 Coexpression Is Uncommon in Sarcomatoid Carcinomas

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Background: Most dedifferentiated liposarcomas (DDLPSs) (~[/underline]90%) are characterized by the coexpression of MDM2 and CDK4, reflecting the coamplification of the encoding genes. This immunoprofile is rarely seen in other high-grade pleomorphic sarcomas (< 5%), and therefore, it has been suggested to be useful for diagnosing DDLPS. Sarcomatoid carcinomas (SCs) may be difficult to distinguish from true sarcomas because the carcinomatous component may be unsampled and

the sarcomatoid element may lack keratin expression. Because SCs often arise from organs in the internal trunk, which is also the favored site for DDLPSs, distinguishing DDLPS from SC can become a diagnostic challenge. However, MDM2 and CDK4 status in SC has not been previously examined, and it is unknown if these markers can help distinguish DDLPS from SC.

Design: We examined 73 SCs arising from various organs in the internal trunk. The tumors tested were, according to the WHO terminology, spindle cell carcinomas of the esophagus (n = 22), carcinosarcoma of the stomach (n = 1), pleomorphic carcinomas of the lung (n = 21), carcinosarcomas of the uterus (n = 24) and ovary (n = 2), and carcinomas with sarcomatoid change of the kidney (n = 3). For each case, immunostaining was performed with antibodies against MDM2 and CDK4. Only the sarcomatoid element was subjected to evaluation. The staining was considered positive when ≥ 1% of tumor cell nuclei were labeled. The results were expressed in terms of intensity (weak or strong) and extent (focal, 1%-10% or diffuse, > 10%).

Results: MDM2 expression was observed in 5/22 (23%) esophageal, 0/1 (0%) gastric, 1/21 (5%) pulmonary, 6/24 (25%) uterine, 0/2 (0%) ovarian, and 2/3 (67%) renal SCs. CDK4 expression was present in 3/22 (14%) esophageal, 0/1 (0%) gastric, 0/21 (0%) pulmonary, 10/24 (42%) uterine, 0/2 (0%) ovarian, and 0/3 (0%) renal SCs. These 2 markers were coexpressed in only 6 (8%) of the 73 cases: 2/22 (9%) esophageal and 4/24 (17%) uterine tumors. In most cases with coexpression, weak or focal reactivity was noted, and none of the cases showed strong diffuse coexpression.

Conclusions: Coexpression of MDM2 and CDK4 in SCs was uncommon (8%), most often, with weak or focal reactivity. These findings are in contrast with those of most DDLPSs that typically exhibit diffuse strong coexpression. Thus, the combination of these 2 markers is likely helpful for distinguishing DDLPS from SC, particularly when the specimen size is limited and conventional epithelial markers produce equivocal results. Ongoing FISH analysis is expected to clarify whether immunoreactivity in SCs reflects underlying gene amplification.

79 Differential Expression of miR-138 in Malignant Peripheral Nerve Sheath Tumors and Desmoplastic Melanomas

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Background: Differentiating malignant peripheral nerve sheath tumors (MPNST) from desmoplastic melanomas (DM) can be a diagnostic challenge due to overlapping morphology and immunophenotype. Treatment regimens and prognosis vary greatly depending on the cancer type. The aim of this study was to identify similarities and differences between these two categories of tumors and potentially find a molecular signature unique to either tumor type as a method of differentiation. MicroRNAs (miRNA) are regulatory molecules that act by means of mRNA inhibition. With practicality and reproducibility in mind, we performed miRNA expression analysis on archived formalin-fixed paraffin-embedded tissue.

Design: A pilot group of 5 MPNST and 3 DM cases with sufficient tissue for miRNA expression analysis was selected. Each case was independently confirmed by a soft tissue pathologist and a dermatopathologist and correlated with follow-up. Blocks containing at least 80% tumor were selected for macrodissection and subsequent total RNA extraction. Samples were quality checked on a Nanodrop for quantity (>1 mg total per 8 uL) and acceptable 260/280 ratio (1.8-2.0). Universal miRNA expression was analyzed with the GeneChip miRNA 3.0 Array (Affymetrix Inc). Raw data was imported into Partek Genomics Suite 6.6 and analyzed using parametric t-test assuming equal variance.

Results: Significant probe sets were identified based on a p-value <0.05 and a significant difference of +/- 2 fold-change between the two groups. Candidate miRNAs showing the most significant differences included miR-138-1/2 (p-value 0.019), miR-452 (p-value 0.044), and miR-4430 (p-value 0.013). See table 1.

Table 1: miRNA Relative Expression in MPNST and DM

Probe	p-value	Relative Fold Expression Change in DM vs. MPNST
hsa-miR-138-1/2	0.0190	-9.61
hsa-miR-452	0.0441	-3.98
hsa-miR-4430	0.0131	2.09

Conclusions: Our preliminary data show, for the first time, that miR-138 is a very promising probe candidate for differentiating MPNST from DM (>9.5 folds expression difference). Although recent studies have suggested a role for miR-138 disregulation in melanoma development, the significance of miR-138 in the pathogenesis of MPNST is unclear and needs further analysis. The other miRNAs (miR-452 and miR-4430) may also be of potential significance. We intend to expand our case selection further and confirm our results using real-time PCR. We believe that miRNA expression analysis, specifically miR-138, can help differentiate and elucidate potential therapeutic targets for MPNST and DM.

80 Aldoketoreductasis 1 B10 (AKR1B10) Expression in Tissues and Tumors Showing Notochordal Differentiation. A Role as a Neoplastic Biomarker and a Potential Therapeutic Target

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Background: Chordoma is a malignant bone tumor which shows notochordal differentiation, sited almost exclusively in vertebrae and base of skull. Being chemoresistant, surgery is the key procedure, although this may be supported by proton-beam therapy. However, given the anatomical location, complete excision is rarely achieved and this accounts for a high incidence of recurrence and late metastasis. Consequently, there is a compelling need for new targeted therapy. It has previously been shown that brachyury, a key gene involved in notochord and chordoma development, indirectly regulates AKR1B10 expression in U-CH1 chordoma cell line. AKR1B10, an enzyme involved in the catabolism of aldehydes and ketones, has been implicated in the development of carcinoma of various organs and in the development of chemoresistance in these tumors. In this study we assessed the expression of AKR1B10 in normal and neoplastic tissues showing notochordal differentiation and in other bone and soft tissue tumors (BSTT).

Design: A TMA of 120 brachyury-positive vertebral chordomas and the full sections of 6 brachyury-positive benign notochordal tumors (BNT) were stained with AKR1B10. To assess the expression of this marker in non-neoplastic notochordal tissues, 10 human embryonic notochords and 2 incidental notochordal rests in intervertebral discs (brachyury-positive) were immunostained with AKR1B10. To assess the brachyury-AKR1B10 link, TMAs including a total of 1,765 BSTT of various types (brachyury-negative), were also tested. Standard immunohistochemistry was performed.

Results: None of the embryonic notochords and notochordal rests showed immunoreactivity for AKR1B10; conversely, all BNT and chordomas were diffusely positive for this marker. Apart from 1/17 Ewing sarcoma and 2/213 myxofibrosarcomas, AKR1B10 was negative in all BSTT.

Conclusions: We show that AKR1B10 is expressed in all benign and malignant notochordal tumors, but not in embryonic notochord or notochordal rests. Nor it is expressed in other BSTT. These findings argue that unlike during embryonic development, in neoplasia brachyury regulates AKR1B10 expression, implicating it in the development of notochordal tumors. Moreover, as AKR1B10 expression is widely reported in a variety of carcinomas and has been associated with chemoresistance, the expression of this enzyme in chordomas may contribute to the chemoresistance observed in these tumors. AKR1B10-inhibitors may therefore have a role in adjuvant therapy.

81 Prognosis of Retroperitoneal Sarcomas Owing to Their Histologic Subtypes: A Large Multicentre Retrospective Analysis Fom the French Sarcoma Group

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Background: Retroperitoneal sarcomas (RPS) are mainly made up of liposarcomas (LPS) and leiomyosarcomas (LMS) that have distinct behaviors. Unclassified pleomorphic sarcomas (UPS) are a heterogeneous group whose proportion in RPS has decreased in time with the recognition that a large part was in fact dedifferentiated LPS. Because of their rarity, these subtypes have been mainly studied and managed as a whole. The aim of this multicentre study was to describe and analyze the behavior and prognosis of the different histological subtypes existing in RPS.

Design: We conducted a retrospective analysis of patients > 18 year-old diagnosed with a RPS in French cancer centers between 01/01/1988 to 31/12/2008.

Results: 313 women and 273 men were included, 57 years-old mean age (range 18-89). The main diagnoses were 244 dedifferentiated liposarcoma (DDLPS, 41.5%), 135 well differentiated liposarcoma (WDLPS, 23%), 109 LMS (18.5%) and 42 UPS (7%). Evolution patterns owing to histology are given in [Table 1]. On multivariate analyses, significant prognostic factors for LR relapse were tumor opening (p=0.01) for WDLPS, expert surgeon (p=0.01) and radiotherapy (p=0.03) for DDLPS. Significant prognostic factors for overall survival were multifocality (p=0.02) and LR involvement (p=0.01) pour WDLPS, grade (p=0.015) for DDLPS, LR involvement (p=0.035) and type of surgery (p=0.018) for LMS.

Evolution Patterns

	WDLPS	DDLPS	LMS	UPS
Nb M0 operated	124	219	83	34
Nb R0/R1 (%)	103 (80.5)	156 (71)	75 (90.4)	21 (61.8)
Nb LR relapse (%)	56 (54)	93 (60)	31 (41)	15 (71)
5-year LRFS	53%	40%	68%	25%
10-year LRFS	21%	24%	55%	-
5-year MFS	96%	86%	47%	73%
5-year OS	82%	61%	61%	35%
10-year OS	63%	31%	30%	-

Nb: Number; M0: metastasis-free; R0/R1: complete resection, LR: Locoregional; LRFS: locoregional relapse-free survival; MFS: Metastasis-free survival; OS: overall survival.

Conclusions: Our results suggest that independent prognostic factors for local control and survival are different among histologic subtypes that may serve as markers for stratifying options of treatment. We demonstrated an impact of the grade on the survival for DDLPS. Overall, the expertise of the surgeon and the avoidance of tumor rupture at first surgery are main prognostic factors, especially for LPS, and underline that initial treatment of these rare diseases in specialized centers should be mandatory.

82 Active TGF-ß Signalling and Decreased Expression of PTEN Separates Angiosarcoma of Bone from Its Soft Tissue Counterpart

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Background: Angiosarcomas constitute a heterogeneous group of highly malignant vascular tumours. Moreover, angiosarcoma of bone is very rare and poorly characterized at the molecular level. Since for angiosarcoma of soft tissue the pathways involved in tumour development and progression are beginning to be explored, our aim is to evaluate the role of these pathways in angiosarcoma of bone.

Design: We collected 37 primary angiosarcoma of bone and used 20 angiosarcoma of soft tissue for comparison. Immunohistochemistry was performed on four TMA slides to evaluate expression of p16, p53, PTEN, BCL2, CDK4, MDM2, Cyclin D1, β-catenin, TGF-β, CD105, phospho-Smad1, phospho-Smad2, HIF-1α, PAI-1, VEGF, CD117 and GLUT-1. PIK3CA, was screened for hotspot mutations in 13 angiosarcoma of bone and 6 angiosarcoma of soft tissue.

Results: In nearly 55% of the angiosarcoma of bone the Rb pathway was affected, either through p16, Cyclin D1 or both. Loss of p16 expression was associated with a significantly worse prognosis. No overexpression of p53 or MDM2 was found, suggesting that the p53 pathway is not important in angiosarcoma of bone. Angiosarcoma of bone showed highly active TGF-β signalling; especially compared to angiosarcoma of soft tissue, with immunoreactivity for phospho-Smad2 and PAI-1. While the PI3K/Akt pathway seems to be active in both angiosarcoma of bone and soft tissue, different mechanisms were involved: 40.7% of angiosarcoma of bone showed a decrease in expression of PTEN, while in angiosarcoma of soft tissue overexpression of KIT was found in 89.5%. PIK3CA hotspot mutations were absent.
Conclusions: In conclusion, the Rb pathway is involved in tumourigenesis of angiosarcoma of bone. The PI3K/Akt pathway is activated in both angiosarcoma of bone and soft tissue, however with a different cause; PTEN expression is decreased in angiosarcoma of bone, while angiosarcoma of soft tissue show overexpression of KIT. Our findings support that angiosarcomas are a heterogeneous group of vascular malignancies, while both angiosarcoma of bone and soft tissue may benefit from therapeutic strategies targeting the PI3K/Akt pathway, interference with TGF-β signalling may be specifically relevant in angiosarcoma of bone.

83 Malignant Peripheral Nerve Sheath Tumor Development Is Associated with PTEN Loss and Activation of the PI3K/AKT/mTOR Pathway

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Background: Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant inherited disorders (incidence 1:2,500) and typically causes benign peripheral neurofibromas (NF). In comparison to the general population, NF patients have a 100-fold lifetime risk of developing malignant peripheral nerve sheath tumors (MPNST). The signaling pathways that underlie the pathogenesis of NF development and malignant transformation are poorly understood. In a previous study, we demonstrated the importance of the PTEN (phosphatase and tensin homologue) tumor suppressor gene as a negative regulator of PI3K/AKT/mTOR pathway in the pathogenesis. Specifically, the loss of expression of PTEN, in combination with K-RAS activation in our mouse model, led to development of NFs with 100% penetrance, followed by MPNST transformation. The purpose of this study was to assess PTEN expression and activation of the PI3K/Akt/mTOR pathway by immunohistochemistry (IHC) in human PNSTs.
Design: PTEN and p-S6 IHC was performed on a peripheral nerve sheath tumor tissue microarray (TMA) containing 42 MPNST cases (35 primary tumors, 4 recurrent, and 3 metastatic), including both spontaneous (n=25) and NF1-associated (n=17) cases. The TMA contains triplicate cores from each case. PTEN (cytoplasmic) and p-S6 (nuclear) staining of tumor cells was scored according to cellular density: 0 staining, <1% of tumor cells; 1+ staining 1-25%; 2+ staining 26-75%; and 3+ staining >75%.
Results: Loss of PTEN expression (less than 1+) was seen in 12/25 (60%) of spontaneous and 8/17 (47%) NF-1 associated MPNSTs. A concomitant upregulation of p-S6 positivity also was seen in 10/12 (83%) spontaneous and 4/8 (50%) NF-1 associated MPNSTs.
Conclusions: PTEN loss occurs frequently in human MPNSTs and correlates with changes in the PI3K/Akt/mTOR pathway, as seen by increased expression of p-S6. This data confirms our prior studies and suggests therapeutic inhibition of this pathway may be useful in both spontaneous and NF1-associated MPNSTs.

84 Immunohistochemical Analysis of Expression of RB1, CDK4, CHMP2B, cPLA2G4A and HSP90 in Myxofibrosarcoma and Myxoid Liposarcoma

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Background: Distinction of myxofibrosarcoma from myxoid liposarcoma can be difficult because of overlapping morphological patterns. This study addresses the diagnostic efficacy and possible role of gene products that have been shown to display varied cellular functions in liposarcoma and other tumors. Cell cycle regulators RB1 and CDK4 are overexpressed in well-differentiated and dedifferentiated liposarcoma. CHMP2B is a core component of endosomal sorting complex required for transport (ESCRT-III) that has been shown to be implicated in myxofibrosarcoma and dedifferentiated liposarcoma. cPLA2G4A belongs to group IV phospholipase A2 family and contributes to tumor progression and angiogenesis in some epithelial neoplasia. HSP90 is a molecular chaperone involved in signal transduction, protein folding, and conformation.
Design: Formalin fixed paraffin blocks from 32 myxofibrosarcoma patients and 29 myxoid liposarcoma patients were accrued following approval by our IRB. All initial diagnosis were confirmed by fluorescence in situ hybridization for CHOP. Immunohistochemical stains for RB1, CDK4, CHMP2B, HSP90 and cPLA2G4A were performed on each case with appropriate negative and positive controls. Only tumors with moderate to strong nuclear and /or cytoplasmic positivity in greater than 30% of the tumor cells were evaluated.
Results: HSP90 demonstrated strong nuclear stain in all liposarcoma cases. Only 4 myxofibrosarcomas showed scattered positivity for HSP90. All but 4 cases of myxofibrosarcoma displayed strong diffuse or focal positivity for cPLA2G4A and CHMP2B stains. In myxoid liposarcoma, cPLA2G4A was positive in only 2 cases with round cell component and both were CHMP2B negative. 8 out of 29 myxoid liposarcoma cases were positive for CDK4, while 15 out of 32 myxofibrosarcoma cases were CDK4 positive. All cases, including high grade tumors, were negative for RB1.
Conclusions: HSP90 and cPLA2G4A may be useful in distinguishing myxofibrosarcoma from myxoid liposarcoma (sensitivity = 100% and 81.3%, specificity = 87.5% and 93.1%, $p<0.00000001$ and $=0.00000001$, respectively). There is loss of RB1 and compensatory CDK4 expression in both myxoid liposarcoma and myxofibrosarcoma. Overexpression of cPLA2G4A or CHMP2B could indicate high grade tumor ($p=0.044$) and are perhaps involved with tumor progression. cPLA2G4A, HSP90 and CHMP2B may also represent potential molecular targets for novel therapeutic approaches.

85 Identification of Novel *PAX3-MAML3* Fusion Gene in Low Grade Sinonasal Sarcoma with Neural and Myogenic Features by Transcriptome Analysis

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Background: Low-grade sinonasal sarcoma with neural and myogenic features (LGSS) is a recently described spindle cell sarcoma of the sinonasal tract characterized by concomitant neural and myogenic differentiation (Lewis JT et al., 2011). In this study we show that the fusion gene *PAX3-MAML3* due to the translocation t(2;4)(q37.1;q31.3) is a recurrent and specific molecular finding of these tumors.
Design: Twenty-two LGSS cases were retrieved from Mayo Clinic tissue archives and referring institutions. The histologic features and immunohistochemical profile of all cases were reviewed for diagnostic confirmation. Cytogenetic analysis was performed on two examples in which fresh tissue was available. RNA was extracted from cultured cells and cDNA libraries were prepared using TruSeq RNA Sample Prep Kit (Illumina Inc, San Diego). Global transcriptome sequencing analysis was performed using HiSeq 2000 sequencer (Ilumina Inc). Potential fusion genes were investigated using the SnowShoes-FTD algorithm for paired end mRNA-Seq data (Asmann Y et al., 2011). High throughput sequence results were confirmed at the genomic level by FISH, and at the transcriptional level by RT-PCR and direct Sanger sequencing on available FFPEs. Fifteen distinct tumors and normal tissues were used as negative controls.
Results: Global transcriptome sequencing analysis identified the fusion of *PAX3* exon 7 to the mastermind-like 3 (*MAML3*) exon 2 in one index tumor with t(2;4). FISH and RT-PCR analyses confirmed the *PAX3-MAML3* fusion in 14 (of 22 cases; 63%). Furthermore, *PAX3* rearrangements only (without *MAML3* involvement) were found in 7 cases (32%), consistent with the presence of alternate *PAX3* fusion genes. A single case showed *MAML3* rearrangement only. All controls, including six alveolar rhabdomyosarcomas, were negative for this novel fusion.
Conclusions: Our study identified the novel *PAX3-MAML3* as a recurrent fusion gene event in LGSS. *PAX3* rearrangement without *MAML3* rearrangement was identified in a subset of cases, implying the presence of alternate *PAX3* fusion genes. The structure of PAX3-MAML3 fusion protein is predicted to retain the paired box and homeobox DNA binding domains of PAX3 and the transactivation domain of MAML3. However, it is still unclear whether the developmental roles of PAX3 and MAML3 may explain the biphenotypic profile of LGSS. Finally, the identification of *PAX3-MAML3* can be used as a novel diagnostic biomarker for LGSS.

86 Differential MicroRNA Expression in Low-Grade and High-Grade Cartilaginous Tumors

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Background: Tumor grade is considered to be the most important prognostic indicator for cartilaginous tumors. However, as there is no univerally accepted grading system, traditional grading can be subjective. We sought to identify more objective markers using miRNA profiling in decalcified formalin-fixed paraffin embedded tissue.
Design: Twenty-nine cartilaginous tumors with decalcified FFPE tissue were identified. Low grade cartilaginous tumors (n=14) included enchondromas (n=5) and low grade chondrosarcomas (n=9). High-grade cartilaginous tumors (n=14) included high-grade chondrosarcomas (n=9) and dedifferentiated chondrosarcoma (n=5). MiRNA expression profiling was performed using a real-time PCR based platform on Taqman Human miRNA microfluidics cards which contain 378 annotated miRNAs. Differential fold change and the student t-test was used on a probe-by-probe basis to compare low-grade verse high-grade cartilaginous tumors.
Results: A set of 57 microRNAs were found to be differentially expressed between low-grade and high-grade tumors ($p<0.05$). 56 of these microRNAs were overexpressed in the high grade cartilaginous tumors while miR-486 was the only microRNA found to be highly expressed in the low grade cartilaginous tumors. Differential expressed miRNAs was also seen between enchondromas, low-grade Chs, high-grade Chs and dedifferentiated Chs.
Conclusions: Our studies indicate that microRNA analysis on decalcified formalin fixed paraffin embedded tissue shows promise as a useful tool for classifying cartilaginous tumors. We identified a set of microRNAs that are differentially expressed between high grade and low grade cartilaginous tumors. Several of the miRNAs overexpressed in the high grade tumors such as miR-21, miR-31, miR-221, miR-222 have been reported to be associated with poor prognosis in other malignancies. Among the reported mRNA targets of miR-21, are PTEN, PDCD4 and NF1B, suggesting a role for miR-21 in regulating apoptosis, proliferation and migration. In addition, low grade tumors had higher expression of miR-486, which is reported to act as tumor suppressor in gastric cancers, perhaps behaving in a similar function in cartilaginous tumors.

87 Myxoid Liposarcoma with Heterologous Components: Dedifferentiation or Metaplasia? A FISH and CGH-Array Documented Case Report

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Background: Heterologous differentiation in myxoid liposarcoma (MLS) is a rare phenomenon. Few cases were thus far reported, often without molecular assays, and the concept of "dedifferentiated" myxoid liposarcoma remains controversial.

Design: We describe a primary MLS with malignant chondroid and osseous components that affected the right thigh of a 27-year-old woman. We wondered if these areas represent dedifferentiated components or simply metaplasia, and performed FISH and CGH-array assays in the myxoid liposarcoma component and in the chondroid component.

Results: FISH analysis demonstrated a *DDIT3* gene rearrangement in the two components, and CGH-array analysis did not detect any gain or loss of DNA regions, neither in the myxoid liposarcoma component, nor in the chondroid component. Thus, our results demonstrated that the two components have the same molecular alterations, and particularly, the heterologous component did not demonstrate additional ones.

Conclusions: Our FISH and CGH-array documented case suggest that the heterologous components seen in some myxoid liposarcomas reflects rather a metaplastic phenomenon than a true dedifferentiation phenomenon, challenging the concept of "dedifferentiated myxoid liposarcoma."

88 MicroRNA-199b-5p Is Involved in Notch Pathway of Osteosarcoma

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Background: MicroRNAs (miRNA) play important roles in the development, differentiation and function of various cell types, and in the pathogenesis of various human diseases. The miRNAs are differentially expressed in normal cells and cancer cells. An investigation of miRNA expression between normal subjects and patients with osteosarcoma is a crucial step for future clinical trials.

Design: We performed a miRNA microarray analysis on 8 formalin fixed paraffin embedded osteosarcoma tissue samples. And we confirmed the result of microarray by reverse transcription polymerase chain reaction.

Results: The data from miRNA profiling of osteosarcoma indicated that 10 miRNAs showed 10-fold increased expression compared to normal control. Among the 10 miRNAs, three miRNAs (miR-199b-5p, miR-338-3p, and miR-891a) were confirmed with reverse transcription polymerase chain reaction. And through further cell line study, we identified that miR-199b-5p influenced the Notch pathway in osteosarcoma. After four osteosarcoma cell lines were transfected with miR-199b-5p inhibitor, the expressions of Notch pathway components (Dll1, JAG1, HES1, Dtx1) in transfected cell lines were changed in comparing with negative controls.

Conclusions: These results revealed that miR-199b-5p was involved in the Notch pathway of osteosarcoma. Recent study suggests that Notch and HES1 signaling may be a therapeutic target to prevent metastasis for human osteosarcoma patients using GSI (γ-secretase inhibitor). Taken together with our results, we suggest that miR-199b-5p inhibitor also may be a therapeutic option in osteosarcoma.

89 Changing Prognostic Factors in Osteosarcoma: Analysis of 381 Cases from Two Institutions

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Background: Osteosarcoma (OSA) occurs most commonly in children/young adults, with a historic second incidence peak in the elderly. Most studies have focused on those occurring in adolescence. Detailed information on descriptive features and prognostic factors in patients of different age groups is lacking. The aim of this study was to identify clinicopathologic factors significantly associated with survival in young, middle, and older age groups.

Design: The tumor registries of the authors' institutions were searched to identify OSA cases between 1973 and 2012. The clinicopathologic factors were recorded, including age, sex, race, tumor site, type, grade and size, and % necrosis post-chemotherapy. Analyses of overall survival (OS) and recurrence free survival (RFS) were performed in each stratified age group (G1, <25, n=202; G2, 25-54, n=118; G3, >54, n=61) by utilizing the Cox proportional hazard model.

Results: A total of 381 OSA cases were retrieved. The peak incidence was in the second decade (41%), and 53% were younger than age 25. There was a steady incidence rate (10%/decade) thereafter until the 6th decade, when it started to decline. There were significant differences in tumor types (conventional vs. other) and sites (long bone vs. other, bone vs. extraskeletal) among different groups. Univariate analysis revealed that factors significant for OS were gender, tumor site, grade and size in G1, tumor type in G2, and tumor size in G3, whereas significant factors for RFS were tumor site and size in G1, tumor type in G2, and race and tumor size in G3. Moreover, % necrosis was a significant factor for RFS only when all age groups were combined, but not for OS. Upon multivariate analysis, factors significantly associated with a favorable OS were female gender ($p<0.05$), long bone location ($p<0.01$), low grade ($p=0.01$) and small tumor size ($p<0.0001$) in G1, parosteal OSA in G2 ($p<0.01$), and extraskeletal site in G3 ($p=0.03$); whereas factors significantly associated with favorable RFS were female gender ($p<0.05$) and small tumor size ($p<0.001$) in G1; and low grade in G2 ($p=0.05$).

Conclusions: Modern OSA did not have a second incidence peak. Rather, it showed a steady incidence rate in middle age individuals and gradual decline in the elderly. There were significant different distributions in tumor types and sites across age groups. Importantly, while %necrosis did not appear to be associated with OS, distinctive prognostic factors differed significantly among different age groups, thus providing a rationale for age-based management strategies.

90 Sensitivity and Specificity of USP6 Gene Break-Apart Assay and Protein Expression in Nodular Fasciitis

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Background: Nodular fasciitis (NF) is a common benign mesenchymal lesion that may progress through myxoid, cellular, and fibrous phases. The differential diagnosis of NF includes many other spindle cell neoplasms and it may be misdiagnosed as a sarcoma due to its rapid growth and active mitotic activity. Recently, it was discovered that the entire coding region of ubiquitin-specific peptidase 6 (USP6), an oncogene translocated in aneurysmal bone cyst (ABC), is fused to the MYH9 promoter region in up to 92% of NFs. This finding indicates a potential application of USP6 gene expression or gene break-apart as a biomarker of NF. However, the sensitivity and specificity of USP6 as a biomarker for nodular fasciitis is not known.

Design: USP6 protein expression was detected by immunohistochemistry (IHC) with a polyclonal antibody (1:20, Sigma-Aldrich) and the USP6 gene break-apart associated with the translocation was tested by fluorescence *in situ* hybridization (FISH) with a DNA probe (Empire Genomics, Buffalo, NY). The assays were performed on microarrays of paraffin embedded tissues composed of NFs (7) and other soft tissue spindle cell neoplasms including desmoid-type fibromatosis (3), solitary fibrous tumors (5), dermatofibromas (2), dermatofibrosarcomas protuberans (DFSP) (3), myositis ossificans (8), neurofibromas (4), schwannomas (3), angioleiomyomas (3), leiomyosarcomas (LMS) (2), low grade fibromyxoid sarcoma (LGFS) (6); and ABCs (11) (9 primary and 2 secondary).

Results: IHC for USP6 shows weak to moderate reactivity in 6 of 7 (86%) NFs; strong reactivity in 9 of 9 (100%) primary ABCs; variable reactivity in 5 of 6 (83.3%) LGFMSs, 3 of 3 (100%) DFSPs, 2 of 2 (100%) leiomyosarcomas, and 2 of 2 (100%) secondary ABCs. FISH for USP6 was positive in 2 of 7 (28.5%) NFs and negative in all other spindle cell neoplasms (39), including 5 cases of LGFSs and 3 cases of DFSPs that were positive by IHC. Only 3 of 11 ABCs had enough material left in tissue microarrays to perform FISH, 1 of 2 primary ABCs was positive and 1 secondary ABCs was negative. The sensitivity and specificity of USP6 for the diagnosis of NF is 86% and 20% and 29% and 100% for IHC and FISH, respectively.

Conclusions: Despite its low sensitivity, the use of a USP6 gene break-apart rearrangement FISH probe seems highly specific for NF and could potentially be used as an aid in its diagnosis. In contrast, USP6 IHC seems highly sensitivity but not specific for NF and its use as an ancillary test in the diagnosis of NF is not warranted.

91 Fascin-1 Overexpression and miR-133b Down-Regulation in the Progression of Gastrointestinal Stromal Tumor

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Background: MicroRNAs (miRNAs) are small, non-coding RNAs that are up- or down-regulated in several types of cancer, and play an important role in the tumorigenesis and progression. The aim of this study is to better understand the role of aberrantly expressed miRNAs and their target genes affecting the biology of gastrointestinal stromal tumor (GIST).

Design: We performed miRNA array in 19 cases of GIST, and subsequently, we examined the expression levels of *miR-133b* and *fascin-1* by quantitative real-time RT-PCR and immunohistochemical staining.

Results: By miRNA array, several miRNAs, including *miR-133b*, were down-regulated in high-grade GISTs as compared with low to intermediate-grade GISTs. Subsequently, quantitative real-time RT-PCR revealed that *fascin-1* mRNA was up-regulated in accordance with *miR-133b* down-regulation in high grade GIST; this result was consistent with a previous functional study showing that *fascin-1* might be a direct target of *miR-133b* in carcinoma. We then examined the fascin-1 protein expression by immunohistochemical staining in 147 cases of GIST, and found that fascin-1 overexpression was significantly correlated with shorter disease-free survival time and several aggressive pathological factors, including tumor size, mitotic counts, risk grade, blood vessel invasion and mucosal ulceration.

Conclusions: Our results suggest that down-regulation of *miR-133b* and overexpression of fascin-1 may play an important role in the progression of GIST, and that fascin-1 may be a useful biomarker to predict the aggressive behavior.

92 Clinical and Biological Significance of Hepatoma-Derived Growth Factor in Ewing Sarcoma/PNET

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Background: To investigate the clinical significance of hepatoma-derived growth factor (HDGF) in Ewing sarcoma/peripheral primitive neuroectodermal tumours (PNET).

Design: Immunohistochemistry staining was used to detect HDGF and p53 expression in 108 patients with Ewing sarcoma/PNET. Statistical analyses were applied to test the prognostic and diagnostic associations. The cell proliferation, apoptosis, G1/S transition of cell cycle, migration and invasion, and colony formation of HDGF downregulation in Ewing sarcoma cell was determined.

Results: Our results showed that fifty-five cases (50.9%, 55/108) showed high HDGF expression in Ewing sarcoma/PNET. Seventeen cases (15.7%, 17/108) were p53 positivity. There were significant relationships between HDGF expression and tumor size ($p=0.008$), metastatic status at initial diagnosis ($p=0.027$), and p53 expression ($p=0.022$). Furthermore, there was a positive correlation between high HDGF expression and p53 positive expression in Ewing sarcoma/PNET($p=0.022$). Patients with high HDGF

expression had shorter overall survival and disease-free survival at 3 or 5 years than those with low HDGF expression, respectively. In addition to distant metastasis, multivariate analysis demonstrated that HDGF expression was an independent strong predictor for OS and DFS in Ewing sarcoma/PNET patients ($p=0.002$, $p<0.001$, respectively). HDGF knockdown dramatically inhibited proliferation, migration, invasion, colony formation and G1/S transition of cell cycle, but induced apoptosis in Ewing sarcoma cells.
Conclusions: In conclusion, our results firstly suggest that nuclear HDGF expression is a significant adverse prognostic factor for overall survival and disease-free survival in patients with Ewing sarcoma/PNET. Targeting HDGF may be a novel strategy for Ewing sarcoma/PNET treatment.

93 Fibromatosis of the Breast: Complication of Previous Radiation and Surgery

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Background: Fibromatosis of the breast is uncommon, and comprises less than 0.2% of all breast tumors. It is a locally aggressive neoplasm and often recurs. Fibromatosis harbors mutations in the beta catenin gene and predisposing factors include Gardner syndrome and a history of previous surgery. Herein, we report five cases of fibromatosis of the breast in patients with a history of prior surgery and radiation.
Design: Departmental and consultation files were searched from 2003 -2012 for cases diagnosed as "desmoid" or "fibromatosis" arising in the breast. Eleven patients with pathologically confirmed breast desmoid tumor were identified. Records were retrospectively reviewed.
Results: Among those eleven patients, one patient had Gardner syndrome, five patients had a history of surgery for breast cancer, two patients had a history of surgery for bilateral breast augmentation, and the remaining three had no documented surgery prior to the development of fibromatosis. Among the seven patients who had surgery, five of them, including one of the patients with breast augmentation, received radiation therapy before developing fibromatosis. The five patients were female and ranged in age from 36-57 (mean of 48.8) years. The fibromatosis tumors presented as palpable masses, clinically and radiographically suspicious for carcinoma. Four patients had a history of breast cancer treated with surgery and radiation (6 weeks of 6040 CGY). One patient had multiple bilateral breast augmentations and had superficial electron bean radiation for cosmetic reasons to prevent scar formation. Fibromatosis was diagnosed at a median of 28 months post-radiation therapy. The tumors ranged from 2.7 cm to 5 cm. All of the patients had their tumor resected and 1 patient had a local recurrence 8 months after the initial excision with positive margins. The remaining patients were disease free at last follow up (0 to 9 years). Microscopically, the tumors showed the classic features of fibromatosis. There was no significant cytologic atypia, pleomorphism or necrosis. Immunohistochemically, the tumor cells expressed beta-catenin in all cases.
Conclusions: The development of fibromatosis is a well recognized complication of different types of therapeutic interventions. Fibromatosis of the breast is uncommon and should be considered when a new mass develops in the breast following surgery and/or radiation. Excision of the mass with or without adjuvant medical therapy should be considered as possible forms of therapy.

94 Primary Malignant Myoepithelial Tumor of Bone

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Background: Primary malignant myoepithelial tumor of bone is a rare malignancy which can resemble morphologically a variety of different tumors including chordoma, myxoid chondrosarcoma, and carcinoma. To increase our understanding of the clinicopathologic features of this unusual neoplasm we reviewed our experience with 6 cases.
Design: The study cohort was identified from the authors consult files and from the surgical pathology files of Massachusetts General Hospital and University of Miami Hospital. The clinical and pathological features of the tumors including their histologic, immunohistochemical, and EWSR1 status were analyzed.
Results: The patients included 3 females and 3 males who ranged in age from 18-57 (mean of 41.3) years. The tumor location included: 1 in the maxilla, 1 in the distal tibia, 1 in the calcaneus, 2 in the mandible, and 1 in the distal femur. Imaging studies showed that the tumors were lytic with cortical destruction and extra-osseous extension. Microscopically, four cases were composed of clusters of large epithelioid and spindle cells enmeshed in a prominent basophilic myxoid stroma. The other two cases were hypercellular and composed of clusters of epithelioid cells and spindle cells with solid and reticular pattern. The tumor cells demonstrated pleomorphism, and high mitotic activity and variable amounts of necrosis. The tumors also infiltrated into the surrounding soft tissue. Immunohistochemically, the tumor cells were focally positive for either S100/ or SMA and EMA/or Keratin. Four cases were analyzed by FISH for detection of *EWSR1* gene rearrangement and one case had a rearrangement, one tumor demonstrated monosomy of chromosome 22, one case was inconclusive, and one case was negative. All patients underwent wide resection.
Conclusions: Primary malignant myoepithelial tumor is a rare bone tumor, is heterogenous morphologically, and can be confused with a variety of different neoplasms. Immunohistochemically these tumors usually express S100 and EMA and have genetic aberrations involving chromosome 22. As very few of these tumors have been described their optimal treatment and natural history needs to be better defined.

2064 Beta-Human Chorionic Gonadotropin Expression in Giant Cell Tumors

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Background: Giant cell tumors are benign yet locally aggressive neoplasms of bone and tendons with a high incidence of recurrence after surgical resection. Additionally, they have the potential to metastasize to distant sites such as the lung and lymph nodes, and the diagnosis can be difficult to establish histologically in this setting. The beta subunit of human chorionic gonadotropin (hCG) is expressed in benign and malignant syncytiotrophoblasts, several non-gynecological neoplasms, and is commonly used as a serum marker in diagnosis and therapeutic monitoring of the associated gynecological tumors. Furthermore, due to the young age of patients with giant cell tumor of bone, urine beta-hCG is often performed to evaluate for pregnancy during the work-up of a lesion. At our institution, we have observed multiple cases of elevated urine beta-hCG in patients with giant cell tumors, which has led to diagnostic difficulty and in one instance, concern for metastatic choriocarcinoma. The aim of this study is to determine if giant cell tumors of bone express beta-hCG, and if so, at what frequency.
Design: We evaluated 15 cases of giant cell tumors consisting of 11 recurrent neoplasms and 4 metastatic lesions. The neoplasms were analyzed for expression of beta-hCG by immunohistochemistry using the Dako polyclonal rabbit beta-hCG antibody and staining was reviewed independently by 2 pathologists.
Results: 40% of cases (n=6) exhibited staining for beta-hCG. 13% had weak (1+) staining, while 20% demonstrated moderate staining (2+), and 7% had strong staining (3+).
Conclusions: Beta-hCG is frequently expressed by recurrent/metastatic giant cell tumors of bone and may be of diagnostic utility when establishing this diagnosis. In addition, it is also important for clinicians and pathologists to be aware of beta-hCG expression by the neoplasm and the potential for elevated urine and serum beta-hCG levels in patients with giant cell tumor of bone so as to avoid misdiagnosis of pregnancy or gestational trophoblastic disease. Currently, the clinical and prognostic significance of beta-hCG expression by giant cell tumors as well as expression by primary tumors is unclear and further studies will address these questions.

Breast

95 The Expression of Cytokeratin 19 in Breast Carcinoma and Its Association with Hormone Receptor Status

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Background: Cytokeratin 19 (CK19) is the lowest molecular weight cytokeratin and is commonly used to mark pancreaticobilliary carcinoma in the liver and papillary carcinoma of the thyroid. CK19 has been shown to be expressed in normal breast tissue and breast carcinoma. CK19 mRNA is also used to detect circulating tumor cells and micro-metastases of breast carcinoma. CK19 expression and its relationship to breast predictive markers (BPM) has yet to be elucidated. The purpose of this study is to identify a relationship, if any, to BPM and other prognostic factors.
Design: Tissue microarrays of 125 cases of varying grades of breast carcinoma had been developed for quality assurance purposes and stained for Estrogen Receptor (ER), Progesterone Receptor (PR), and HER2/neu and scored using techniques prescribed by CAP and ASCO. CK 19 staining was performed and considered positive if any cytoplasmic or membranous stainging was present. Hormone expression was used to classify each tumor into the following subtypes: ER positive/HER2-neu negative (ER+), HER2-neu positive (HER2+) and triple negative (TN). Each subtype was compared to tumor expression of CK19 and reported as a percentage. Prognostic factors such as tumor size, tumor grade, and lymph node status were also compared to CK19 staining. Chi square analysis was performed to identify any significant findings.
Results: Overall expression of CK19 in breast carcinoma was 87.2%. A significant difference was seen when comparing TN to the other subtypes: 98% of (ER+), 88% of (HER2+), and 73% of (TN) expressed CK19 ($\chi^2=14.34$, $p<0.0007$). With regard to tumor grade, 100% of Grade 1, 94% of Grade 2, and 84% of Grade 3 carcinomas express CK19; however, this difference was not significant. An association between tumor size and lymph node status when compared to CK19 expression was not identified.
Conclusions: Cytokeratin 19 is expressed diffusely in breast carcinoma. Our data suggests CK19 expression does not correlate with tumor grade, tumor size, and lymph node status. However, we found a significant number of triple negative breast carcinoma lack CK19 expression. Thus independent use of CK19 may lack sensitivity in detecting metastatic breast carcinoma, specifically in those tumors that lack ER and HER2-neu receptor expression.

96 HER2 Testing: Central Laboratory Assessment of HER2-Equivocal (2+ Staining) Samples Using IHC and Dual SISH

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Background: In breast cancer overexpression or amplification of HER2 is associated with higher metastatic risk and shorter overall survival. In these patients, however, therapies targeting HER2 prolong overall, progression-free and disease-free survival. Immunohistochemistry (IHC) is the method most frequently used to assess HER2 overexpression, but equivocal results (2+) have to be further studied in order to classify them as positive or negative.
Design: In August 2003 a network of Pathology Laboratories trained in HER2 assessment by IHC was set up in Argentina, supervised in turn by an Advisory Board