and testicular atrophy with aspermatogenia (negative OCT3/OCT4 stain). There was evidence of acute multifocal bronchopneumonia and congestive heart failure. He carried two heterozygous mutations in ALMS1: 11316_11319delAGAG; R3772fs in exon 16 and 8164C>T ter; R2722X in exon 10.
Conclusions: This report describes previously undefined cardiac abnormalities in this rare multisystem disorder. Myofibrillar disarray is probably directly linked to ALMS1 mutation, while fibrosis in multiple organs may be a secondary phenomenon to gene alteration. Whether and how intracellular trafficking or related signals lead to cardiac dysfunction is a subject for further research.

45 Sudden Cardiac Death in Young Adults: An Audit of Coronial Autopsy Findings

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Background: Sudden adult cardiac death is in most cases attributable to atheromatous coronary artery disease. Non-atheromatous causes of cardiac death include congenital heart diseases, cardiomyopathies (CM) and valvular heart disease. In recent years the advent of sudden adult cardiac death with a morphologically normal heart (sudden adult death syndrome, SADS) has generated much debate and led to the establishment of registries to investigate these deaths further.
Design: A list of suspected sudden cardiac deaths among young adults (16 – 45 years) was compiled using data from the Central Statistics Office in Ireland from 2004 to 2009. The pathologists and coroners involved in each case were contacted and a copy of the autopsy report requested. The autopsy reports were audited using agreed dataset criteria including demographic factors, toxicology, detailed cardiac parameters, histology and toxicology. The findings were reviewed with regard to cause of death, cardiac pathology and diagnosis of sudden cardiac death.
Results: 90 autopsy reports were received and audited, 16 cases were excluded due to age criteria (≤ 16 years). There were 74 adults, 27 women (36%) and 47 men (64%). The mean age at death was 27.7 years (range 17 – 41 years). The breakdown of the cause of death was as follows: 47 cardiac deaths, 22 non-cardiac deaths, 3 SUDEP and 2 SADS. Coronary artery disease (CAD) was identified in 24 patients (32.4%), however only 26% of these had evidence of IHD. CAD was seen in association with CM in 5 and SADS in 2. Overall, 23 patients (33%) had an enlarged heart (weight > 400g women and 500g men), for which the causes of death included hypertrophic cardiomyopathy (HCM, n=4), dilated cardiomyopathy (DCM, n=3), left ventricular hypertrophy (LVH, n=5), myocarditis (n=1) and valvular disease (n=3) and ischaemic heart disease (IHD, n= 5). Causes of death with a normal heart weight included DCM in 2, ARVD in 1, myocarditis in 2, IHD in 10, and SADS in 8. LVH was seen in that group in 3 cases, but death not attributed to it.
Conclusions: Sudden adult death is a diagnosis of exclusion with important consequences for the living relatives, in the era of molecular diagnosis of genetic cardiomyopathies and channelopathies. Thourough exmination of the heart at autopsy is mandatory in cases of sudden adult death, as SADS is a diagnosis of exclusion, and some cardiomyopathies may present with an apparently normal heart.

46 Recurrent Respiratory Papillomatosis with Malignant Transformation in Lungs: A Retrospective Longitudinal HPV Study

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Background: Recurrent respiratory papillomatosis (RRP) is etiologically associated with vertical transmission during vaginal delivery from an HPV infected mother. Even though cervicovaginal HPV infection is extremely common, RRP occurs in only 4.3 per 100,000 children. Approximately 1-2% of the patients with an early onset of RRP eventually develop laryngeal squamous cell carcinoma (SCC); pulmonary SCC is even rarer. The malignant transformation of RRP to SCC has been linked to various HPV strains and may result from the spontaneous loss of HPV expression. Here we study HPV presence in the early, intermediate and terminal stages of RRP with extensive pulmonary involvement and development of SCC.
Design: Four laryngeal biopsies, obtained at early, intermediate and late stages of disease, as well as autopsy sections were studied by in-situ hybridization with low-risk (types 6/11) and high-risk (types 16/18) HPV DNA probes using Ventana kit on BenchMark XT autostainer with appropriate positive and negative controls. Autopsy was restricted to the chest.
Results: A 13-year-old male underwent 14 excisions of laryngeal papillomas since the age of 2. The lesions caused airway obstruction, frequently complicated by pneumonias and exacerbations of asthma. The patient died from respiratory failure and suppurative bronchopneumonia. Autopsy findings were remarkable for multiple squamous papillomas with viral change and dysplasia in the trachea and both main-stem bronchi. Some papillomas showed in-situ and invasive, focally necrotizing squamous cell carcinoma with vascular invasion and hilar lymph node metastasis. Lung parenchyma uninvolved with tumor demonstrated severe acute bronchopneumonia with microabscess formation. HPV assay showed consistent diffuse strong reaction (>400 viral copies) with low-risk HPV probes in all 4 RRP biopsies, as well as the in-situ and invasive SCC. Low-copy numbers (10-50) of high-risk HPV were detected only in 2 biopsies at the intermediate stage of RRP.
Conclusions: This is a rare instance of early onset RRP with documented progression to dysplastic papillomas, pulmonary SCC in situ and invasive SCC with local metastases. The rarity of RRP, presumed to be acquired during vaginal delivery, is not congruent with the common occurrence of low-risk HPV in the female genital tract, where these viruses are seldom associated with malignant transformation. The HPV typing in this case supports earlier similar reports and suggests an important role of low-risk HPV strains (6/11) in the malignant transformation of RRP in the lower respiratory tract.

47 Comparison of Autopsy Findings of 2009 Pandemic Influenza A (H1N1) with Seasonal Influenza in Four Pediatric Patients

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Background: The swine-origin influenza A (H1N1) virus that emerged in humans in early 2009 has reached pandemic proportions and cause over 120 pediatric deaths nationwide. Studies in animal models have shown that the 2009 H1N1 influenza virus is more pathogenic than seasonal A virus, with more extensive virus replication and shedding occurring the respiratory tract.
Design: We report four cases of influenza A-associated deaths (two pandemic and two seasonal) in persons less than fifteen years of age who had no underlying health issues. Autopsy finding on isolation of virus from various tissue specimen, cocurrent bacterial infection and pathological changes of the respiratory tract were compared between swine-oringin influenza A H1N1 and seasonal influenza infected patients.
Results: The swine-origin influenza A-subtype H1N1 was isolated from post-mortem throat swabs, lung and brain tissue and was confirmed by real time RT-PCR in two cases. Isolates from throat swabs in two other cases were positive for seasonal influenza A virus H1N1 (non-swine) and H3N2, respectively. Evidence of concurrent bacterial infection, S. *aureus* (MRSA), was found in lung and blood specimens in both swine-origin influenza A H1N1 patients. H. *influenzae bacillus* was identified from the throat culture in one of two seasonal flu cases. Examination of the respiratory tract revealed marked hemorrhagic and necrotic changes of upper airway mucosa and hemorrhagic pleural fluid in swine-origin H1N1 patients. Whereas pathology evaluation of postmortem lung specimens showed non-specific edema and congestion in seasonal flu cases, diffuse alveolar damage with prominent hyaline membrane and type II pneumocyte proliferation, hemorrhagic necrosis of bronchiolar walls and neutrophilic infiltration were evident in the lungs of swine-origin H1N1 infected patients.
Conclusions: Our study suggests that bacterial superinfection in lungs and acute respiratory distress syndrome can play a pivotal role in fatal swine-origin influenza A (H1N1) cases.

Bone & Soft Tissue

48 CD1a Immunopositivity in Perivascular Epithelioid Cell Neoplasms (PEComas): True CD1a Expression or Technical Artifact?

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Background: PEComas comprise a family of rare neoplasms composed of morphologically distinctive perivascular epithelioid cells exhibiting a "myomelanocytic" immunophenotype. The distinction of PEComas from other tumors with melanocytic and smooth muscle differentiation can be difficult. A recent study has claimed that PEComas routinely express CD1a, a Langerhans cell-associated transmembrane glycoprotein involved in antigen presentation, and that expression of this marker may be helpful in the distinction of PEComas from various mimics. We evaluated a series of PEComas and potential mimics for CD1a expression.
Design: A total of 54 cases (27 PEComas; 11 leiomyosarcomas; 10 melanomas; 6 clear cell sarcomas) were evaluated in 2 laboratories (Laboratory A: 31 cases, Laboratory B 23 cases). Nine Laboratory B cases were retested at Laboratory A. Laboratory A methods: MTB1 clone (1:20, Novocastra), heat-induced epitope retrieval in EDTA (pH 8.0), Dako Advance detection system (Dako Corp.) with background reducing diluent. Laboratory B methods: MTB1 clone (1:30, CellMarque), heat-induced epitope retrieval in Medium Cell Conditioner #1 (pH 8.0–9.0), streptavidin-biotin detection system with DAB chromogen. Scoring: 1+, 5-25%; 2+, 26-50; 3+, >51%. Langerhans cells served as a positive internal control in all tested cases.
Results: All Laboratory A cases were negative. 16 Laboratory B PEComas (14 renal angiomyolipomas, 1 soft tissue PEComa, 1 pulmonary clear cell "sugar" tumor) showed CD1a immunopositivity (1+: 7 cases; 2+: 7 cases; 3+: 2 cases). All non-PEComas were negative. All positive PEComas showed cytoplasmic staining only, without membranous staining. The 9 Laboratory B positive PEComas were negative when retested at Laboratory A.
Conclusions: We conclude that PEComas do not truly express CD1a in a biologically plausible membranous pattern, but may instead show aberrant cytoplasmic immunopositivity in some laboratories. Close inspection of published photomicrographs of previously reported CD1a-positive PEComas shows an identical pattern of cytoplasmic positivity. This aberrant pattern of immunopositivity likely reflects a technical artifact related to epitope retrieval and detection methods. Alternatively, this staining could represent cross-reactivity with an epitope unique to PEComas, as it was not observed in non-PEComas. Ultimately, however, we do not believe there is a real role for CD1a immunohistochemistry in the differential diagnosis of PEComas.

49 Novel *EWSR1-POU5F1* Fusion in Soft Tissue Myoepithelial Tumors. A Molecular Analysis of 29 Cases, Including Soft Tissue, Bone and Visceral Locations Showing Common Involvement of *EWSR1* Gene Rearrangement

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Background: The diagnosis of myoepithelial tumors (MET) outside salivary glands remains challenging, especially in unusual clinical presentations, such as bone or visceral locations. Few reports have indicated an *EWSR1* gene rearrangement in soft tissue MET, and, in one case each, the fusion partner was identified as being either *PBX1* or *ZNF444*. However, larger studies to investigate if these genetic abnormalities are recurrent or if

restricted to the soft tissue location are lacking.
Design: 29 MET from mainly soft tissue, but also bone & visceral locations, showing classic morphologic features and supporting immunoprofile, with available tissue were included for molecular analysis. Patient age ranged from 1-70 years old. Gene rearrangements in *EWSR1, PBX1* and *ZNF444* were investigated by FISH. In 2 cases with *EWSR1* rearrangement and frozen tissue available, 3'RACE was performed to identify potential novel fusion partners.
Results: *EWSR1* gene rearrangement by FISH was detected in 59% cases. A novel *EWSR1-POU5F1* was identified in a pediatric soft tissue MET by 3'RACE and subsequently confirmed in 2 additional soft tissue tumors in young adults, but not in other locations. The presence of a *EWSR1-PBX1* was seen in one soft tissue tumor, while *EWSR1-ZNF444* was noted in one pulmonary MET.
Conclusions: *EWSR1* gene rearrangement is a common event in MET irrespective of their anatomic location. Many of the *EWSR1* negative tumors were superficial lesions, suggesting the possibility of genetically distinct groups. A subset of soft tissue MET harbor a novel *EWSR1-POU5F1* fusion, which can be used as a molecular diagnostic test in difficult cases.

50 A Subset of PEComas Harbor *TFE3* Gene Fusions

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Background: Perivascular epithelioid cell neoplasms (PEComas) include the common renal angiomyolipoma, pulmonary clear cell sugar tumor and lymphangioleiomyomatosis, and less common neoplasms of soft tissue, gynecologic and gastrointestinal tracts. Recently, aberrant immunoreactivity for TFE3 protein (a sensitive and specific marker of neoplasms harboring *TFE3* gene fusions) has been reported in as many as 80% of PEComas. *TFE3* gene status in these neoplasms has not been systematically investigated, though a recent case report has documented a *PSF-TFE3* gene fusion in an extrarenal PEComa.
Design: We used a fluorescence in situ hybridization (FISH) break-apart assay to evaluate for *TFE3* gene fusions in archival material from 26 PEComas. These cases included 2 previously published TFE3 immunoreactive non-renal PEComas, 12 additional non-renal PEComas (5 soft tissue, 4 abdominal, 2 uterine, 1 hepatic), and 12 renal angiomyolipomas with predominant spindle or epithelioid morphology. Results were correlated with TFE3 immunoreactivity and clinicopathologic features.
Results: Three non-renal PEComas (mean patient age 19 years) demonstrated *TFE3* gene fusions by FISH; all three demonstrated strong positive (3+) TFE3 immunoreactivity. Two of these cases had adequate mRNA for RT-PCR analysis, and neither harbored a *PSF-TFE3* gene fusion. In addition, a metastasis of a uterine PEComa which showed moderate positive (2+) TFE3 immunoreactivity demonstrated *TFE3* gene amplification, a previously unreported phenomenon. None of the other 22 PEComas (mean patient age 54 years) demonstrated *TFE3* gene alterations, though 4 demonstrated moderate positive (2+) TFE3 immunoreactivity. All 4 PEComas with *TFE3* alterations immunolabeled strongly for Cathepsin K, similar to other PEComas.
Conclusions: A subset of PEComas harbor *TFE3* gene fusions. While numbers are small, distinctive features of these cases include young age, extrarenal location, absence of association with tuberous sclerosis (TS), predominant epithelioid clear cell morphology, minimal immunoreactivity for muscle markers, and strong (3+) TFE3 immunoreactivity. Despite significant morphologic overlap with other PEComas, PEComas harboring *TFE3* gene fusions may represent a distinctive entity.

51 Adult Fibrosarcoma (FS): A Reevaluation of 154 Putative Cases Diagnosed at a Single Institution over a 48 Year Period

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Background: Adult FS, defined by the WHO as a "malignant tumor, composed of fibroblasts… and, in classical cases, a herringbone architecture" was once considered the most common adult sarcoma. Currently FS is regarded as a diagnosis of exclusion, representing only 1-3% of adult sarcomas. This trend is likely due to: 1) widespread use of immunohistochemistry (IHC) and molecular diagnostic techniques, 2) recognition of distinct FS subtypes, and 3) distinction of FS from undifferentiated pleomorphic sarcoma (UDPS). However, no recent series has critically re-evaluated putative FS, to estimate their true incidence.
Design: 178 cases diagnosed as adult FS in soft tissue locations were retrieved from our institutional archives for the period 1960-2008. 24 cases with insufficient material were excluded. Based on the morphology of the final 154 cases, IHC was performed using some combination of: wide-spectrum CK, EMA, high molecular weight CK, S100, Melan A, HMB-45, CD34, CD31, TLE1, smooth muscle actin, desmin, Myo-D1, myogenin, c-kit, INI1, calretinin, WT1 and TTF1. Revised diagnoses were based on clinical, morphological, and IHC findings.
Results: The original group of putative FS occurred in 81 M and 73 F (median 53 years, range 2-99 years), and involved the legs (50 cases), head/neck (30 cases), thorax/abdomen (22 cases), arms (21 cases), mediastinum (8 cases), abdomen/ retroperitoneum/ pelvis (13 cases), and lung (10 cases). Only 22 cases met WHO criteria for FS. These occurred in 13 M and 9 F (median 49 years, range 6-74 years), and involved the legs (11 cases), head/neck (6 cases), thorax/abdomen (2 cases) mediastinum (2 cases), and arm (1 case). Non-FS (132 cases) were reclassified as: UDPS (38 cases), synovial sarcoma (21 cases), SFT (14 cases), myxo-FS (10 cases), MPNST (6 cases), DFSP, desmoplastic melanoma (4 cases each), LG fibromyxoid sarcoma, sarcomatoid CA, desmoid, RMS, myofibroblastic sarcoma, spindle cell liposarcoma (3 cases each), sclerosing epithelioid FS, fibroma-like epithelioid sarcoma, leiomyosarcoma, cellular fibrous histiocytoma (2 cases each), and other (9 cases).
Conclusions: Using modern diagnostic criteria and ancillary IHC we have been able to reclassify 87% of putative FS. Exclusive of UDPS, the distinction of which from FS is subjective, 61% of putative FS were reclassified, most commonly as monophasic synovial sarcoma and solitary fibrous tumor. We conclude that true FS is exceedingly rare, accounting for <0.1% of ~18,000 adult soft tissue sarcomas seen at our institution during this time period, and should be diagnosed with great caution.

52 Chondroblastoma-Like Osteosarcoma: A Clinicopathologic Review of 17 Cases

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Background: Chondroblastoma-like osteosarcoma (CBL-like OS) is an exceedingly rare histologic variant of OS that morphologically simulates CBL. Although this subtype is recognized in the WHO classification of bone tumors and bone pathology textbooks, there are only two documented cases in the literature.
Design: We conducted a clinicopathologic and radiographic review of 17 cases collected from 3 large consultative practices of OS showing histologic features resembling CBL. Histologic sections from the primary tumor (all cases) and pulmonary metastases (2 cases) were reviewed. Radiographic information was available on 13 cases.
Results: The tumors occurred in 9 M and 8 F with a median age of 35 years (range, 13-72 years), and involved the following bones: metatarsus (3 cases); femur (3 cases); rib (3 cases); humerus (2 cases); and talus, phalanx, tibia, fibula, ischium and ilium (1 each). In the long bones, tumors arose in the meta-diaphysis (6 cases) and epiphysis (1 case). The constant histologic feature that simulated CBL was the diffuse growth of monotonous, minimally to moderately atypical rounded cells with ovoid nuclei. Cytologic atypia was more pronounced in recurrent tumors. The most helpful feature of malignancy was destructive permeation of host bone/soft tissue. The tumors also showed a variable pattern of abnormal bone production including diffuse sheets of dense osteoid, heavy irregular trabeculae of bone, and coarse or linear calcification of woven bone. The pulmonary metastases had histologic features of OS. 13 tumors had malignant or worrisome radiographic findings, and 1case was equivocal. Of patients with follow-up information, 6 developed one or more local recurrences, 2 developed pulmonary metastases, and 2 died of disease.
Conclusions: We confirmed a subtype of OS resembling CBL. In a tumor with cytologic features similar to CBL, histologic evidence of destructive growth and/or abnormal pattern of bone production should raise the possibility of CBL-like OS, particularly if the tumor is located in an unusual site for CBL or has radiographic findings suggesting malignancy.

53 A Pro-Inflammatory Tumor Microenvironment with High Chemokine Expression and High Tumor-Infiltrating CD3+CD8+ Numbers Associates with Improved Survival in Ewing Sarcoma (EWS)

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Background: Despite multimodal therapy, patients with advanced-stage EWS have a poor prognosis. Immunotherapeutic strategies may provide novel treatment modalities. Although EWS cells can be recognized and targeted by T and NK cells in vitro, limited information is available about tumor-immune interactions within the tumor microenvironment. Here we investigate characteristics of the immune cell infiltrate and chemokine expression in EWS, as well as their mutual relationship and correlations with clinicopathological parameters.
Design: EWS tumors (n=20) were evaluated by quadruple immunohistochemistry for presence and spatial distribution of tumor-infiltrating lymphocytes (TIL; CD3+/CD4+/CD8+). Chemokine expression was analyzed in both tumors and cell lines (n=9) by qRT-PCR, immunohistochemistry and flow cytometry.
Results: Substantial inter-tumor variations were observed for both numbers and distribution (tumor- or stroma-infiltrating) of TIL as well as for chemokine expression profiles. Tumor-infiltrating T-cells contained higher percentages of CD3+CD8+ cells as compared to stroma-infiltrating cells ($p=0.04$). Moreover, survival analysis revealed prognostic benefit of a more robust CD3+CD8+ T-lymphocytic infiltrate ($p=0.04$). Positive correlations between gene expression levels of several functionally related, pro-inflammatory chemokines (mainly CCR5-/ CXCR3-ligands) and TIL numbers ($p<0.05$) suggested that expression of these chemokines contributed to TIL recruitment. The presence of both constitutive and IFNγ-inducible expression of several pro-inflammatory chemokines (CCL5, CXCL9, CXCL10) was confirmed at protein level. High chemokine expression levels, like the presence of high tumor-infiltrating CD3+CD8+ cell numbers, associated with improved overall survival ($p=0.03$).
Conclusions: These results demonstrate prognostic benefit of a tumor microenvironment with high levels of pro-inflammatory chemokines as well as high numbers of tumor-infiltrating CD3+CD8+ T-lymphocytes and point to a role for adaptive anti-tumor immune responses in prevention of tumor progression.

54 Liposarcomas with Mixed Well-Differentiated and Pleomorphic Features – A Clinicopathologic Study of 8 Cases

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Background: Pleomorphic liposarcoma (PL) is defined as an undifferentiated pleomorphic sarcoma containing a variable number of pleomorphic lipoblasts. It is considered a distinct high grade sarcoma unrelated to other forms of liposarcoma since it almost always arises de novo without an associated low grade precursor lesions (e.g. well differentiatied liposarcoma (WDL)) and rarely co-exists with other liposarcoma patterns. We have, however, observed a small number of cases of PL which arose in association with WDL and have studied these cases to define their clinico-pathologic

features and their nosologic relationship to other forms of liposarcoma.
Design: Cases showing the presence of both PL and WDL were retrieved from our consultation archives. The tumors were characterized at the molecular cytogenetic level for the presence of *MDM2/CPM* amplification when archival tumor blocks were available. One hundred cells were scored in each instance by two independent investigators. Follow-up information was obtained from the referring clinicians.
Results: Eight tumors were identified, occurring in 4 men and 4 women (mean age 55 years, range 35-78 years). Sites of origin included the retroperitoneum (3), scrotum (2), buttock (2), and abdominal cavity (1). Tumors consisted predominantly of typical WDL, with an "abrupt" transition to pleomorphic spindle cell sarcoma containing pleomorphic lipoblasts. *MDM2*/*CPM* amplification was present in 4 of 6 (66%) cases, all of which consisted chiefly of PL in the studied blocks. Clinical follow-up (6 of 8 (75%) patients; range 7-29 months; median 19 months) showed 5 patients alive without disease and 1 dead of disease.
Conclusions: PL with WDL is a rare pattern in liposarcoma. In the past such tumors were considered liposarcomas of mixed type to imply two distinct forms of liposarcoma occurring in the same lesion. The presence of *MDM2/CPM* amplification in the PL component of mixed WDL/PL suggest that a subset of PL may arise through tumor progression of WDL or may represent a "transitional" or partially differentiated step toward a classic DL. On-going review of a large cohort of tumors previously diagnosed as "DL" and additional molecular genetic study of both WDL and PL zones should clarify the relationship of these unusual tumors to classical DL, a tumor which by definition does not show lipoblastic differentiation.

55 Recurrent (2;2) and (2;8) Translocations in Rhabdomyosarcoma without the Canonical PAX-FOXO1 Fuse *PAX3* to Members of the Nuclear Receptor Transcriptional Coactivator (*NCOA*) Family

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Background: The fusion oncoproteins *PAX3-FOXO1* [t(2;13)(q35;q14)] and *PAX7-FOXO1* [t(1;13)(p36;q14)] typify alveolar rhabdomyosarcoma (ARMS); however, 20-30% of cases lack these specific translocations.
Design: Cytogenetic and/or molecular characterization to include FISH, RT-PCR and sequencing analyses were conducted on four ARMS and one embryonal rhabdomyosarcoma (ERMS) exhibiting a 2q35 or *PAX3* rearrangement but lacking a *PAX-FOXO1* transcript.
Results: Novel, recurrent t(2;2)(p23;q35) or t(2;8)(q35;q13) translocations fusing *PAX3* to *NCOA1* or *NCOA2* respectively were identified. The *PAX3-NCOA1* and *PAX3-NCOA2* transcripts encode chimeric proteins composed of the paired-box and homeodomain DNA-binding domains of *PAX3,* and the CID domain, the Q-rich region and the AD2 domain of *NCOA1* or *NCOA2*. To investigate the biological function of these recurrent variant translocations, the coding regions of *PAX3-NCOA1* and *PAX3-NCOA2* cDNA constructs were introduced into expression vectors with tetracycline-regulated expression. Both fusion proteins showed transforming activity in the soft agar assay. Deletion of the AD2 portion of the PAX3-NCOA fusion proteins reduced the transforming activity of each chimeric protein. Similarly, but with greater impact, CID domain deletion fully abrogated the transforming activity of the chimeric protein.
Conclusions: These studies: (1) expand our knowledge of *PAX3* variant translocations in RMS with identification of a novel *PAX3-NCOA2* fusion; (2) show that both *PAX3-NCOA1* and *PAX3-NCOA2* represent recurrent RMS rearrangements; (3) confirm the transforming activity of both translocation events and demonstrate the essentiality of intact AD2 and CID domains for optimal transforming activity; and, (5) provide alternative approaches (FISH and RT-PCR) for detecting *PAX-NCOA* fusions in nondividing cells of RMS. The latter could potentially be utilized as aids in diagnostically challenging cases.

56 A Novel Pharmacodynamic Approach To Predict Tumor Response to Targeted Drugs in Sarcoma Patients

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Background: Molecularly targeted therapeutics are designed to interfere with the function of a biological pathway within the cancer cell that is critical to its growth or survival. The optimal development of molecularly targeted anticancer agents is limited by the lack of clinically applicable tools to predict drug effects. This study aimed to develop methods that might be useful in predicting the efficacy of targeted agents in sarcoma cells obtained from patient tumors.
Design: All tissue studies were conducted in accordance with IRB requirements. Tumor cells were exposed to drugs targeting IGFR, Met, PDGFR, MEK, AKT and mTOR in short-term cell culture conditions (*ex vivo*). Cellular extracts were prepared and the expression levels and phosphorylation status of intracellular signaling proteins as well as markers of apoptotic cell death (PARP cleavage and caspase 3 activation) and cell proliferation (phospho-histone H3) were analyzed in triplicate by using the Luminex® xMAP system.
Results: Tumor cells obtained from patients with malignant peripheral nerve sheath tumor, osteosarcoma, undifferentiated sarcoma, malignant gastrointestinal stromal tumor and dedifferentiated liposarcoma provided sufficient amount of viable tumor cells to perform short term *ex vivo* pharmacodynamic assays. The quantitative multiplex assays enabled the simultaneous analysis of the activation status of the membrane tyrosine kinase receptors, their downstream intracellular signaling pathways as well as drug-mediated changes in the cell proliferation and apoptotic cell death in sarcoma cells. Multiplex analysis demonstrated significant variation in the molecular response of tumor cells collected from sarcoma patients to the targeted drugs where target inhibition frequently did not correlate with inhibition of the downstream signaling pathways, suggesting the existence of additional compensatory mechanisms.
Conclusions: Multiplex approach is versatile and highly sensitive for the simultaneous analysis of the multiple components of intracellular signaling pathways in small tumor samples to predict and assess the pharmacodynamic efficacy of targeted agents in tumor samples. This approach may offer a means of enriching clinical trials to better identify effective candidate regimens for patients with given tumor types. Ultimately, if validated in clinical trials, tools such as these may afford a means of tailoring the most efficient therapeutic regimen for individual patients.

57 Gene Expression Profile of Chemotherapy Effect on Human Ewing Sarcoma Cell Lines

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Background: Multimodality therapy includes surgery, radiation and chemotherapy/neoadjuvant chemotherapy is the cornerstone of current treatment of Ewing Sarcoma (ES). Chemotherapy regimens usually include drugs such as doxorubicin (antitumor antibiotic), etoposide (topoisomerse II inhibitor) and 4 hydroperoxycyclophosphamide (4-HC), ifosfamide or cyclophosphamide (alkylating agent). Understanding the molecular mechanism of drug effect is important in developing best therapeutic regimens to minimize drug resistance. Herein, we investigated the gene expression profile of ES cell lines subjected to chemotherapies with single and multiple agents.
Design: Three human ES cell lines (A673, SKES and RDES) each were incubated without any agent, with single agent, or with combination of doxorubicin (dox), etoposide (etop) and 4-HC at the concentration killing 30% of the cells in 24 hrs. The extracted RNA was then subjected to Sentrix BeadChip Array HumanHT-12_v3_ BeadChip (Illumina, San Diego, CA) for gene expression analysis with appropriate quality and reproducibility controls.
Results: RNA of all experimental groups except A670 with combination treatment were sufficient for profiling. Signature genes for ES (such as EWS, FLI1, ETV1 and ZSG) were present. Out of 38,000 genes evaluated, 13, 913 genes probes were expressed in most of the samples (8 or more). Fifty-eight probes showed significant change in expression after treatment with each drug (t-test, $p<0.05$). Among them RPS20, RPL8, and RPS25 were up regulated up to 2 folds; while CPT1B, C11OKF47 and KIF27 were down regulated up to 5 folds. Combination drug therapy revealed up-regulation up to 4 folds and down-regulation up to 18 folds.
Conclusions: Significant differential gene expression (especially 58 genes) was seen in ES cell lines subjected to single and combo drug therapy compared to controls. Enhanced effect on specific gene expression by combination therapy suggests an addictive/synergistic antitumor effect due to regulation of similar molecular signatures. These finding may aid in design of future studies of clinical samples and larger series for predicting and monitoring chemotherapy response and for identificaition of potential molecular targets.

58 Multifacted Morphological Evaluation of High-Risk Soft Tissue Sarcoma Pre and Post Combination Neo-Adjuvant External Beam Radiation (EBRT) with Intratumoral Injection of Dendritic Cells Immunotherapy

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Background: Patients with large (>5 cm), high grade soft tissue sarcomas (STS) have a high (>50%) risk of systemic disease. Herein, we report a prospective trial that adds innovative immunotherapy to conventional therapy by using neo-adjuvant external beam radiation (EBRT) with experimental intratumoral injection of dendritic cells (DC). Our hypothesis is that DC will enhance anti-autologous tumor cell immune responses. To elucidate the effectiveness and mechanism, this study determined both the therapy effect and T lymphocyte / DC immune response by morphological evaluation of formalin-fixed and paraffin embedded tumor sections.
Design: Eighteen STS of extremity/trunk/chest wall (stage T2N0M0 or T3N0M0) were treated with standard neo-adjuvant EBRT 5040 cGy coordinated with DC therapy. Histological examination of pre and post therapy H&E slides was to confirm diagnosis, evaluate therapy effect including % of tumor necrosis, and evaluate the presence of lymhocytes and histiocytes. Immunohistochemical studies of CD3, CD4, CD8 and CD68 stains were undertaken to further characterize the immune response; the number of T cells and histiocytes were counted in ten x200 fields chosen from the most mitotically active areas of the tumor by two independent reviewers.
Results: Eighteen STS included myxoid sarcoma (3), synovial sarcoma (2), scelrosing epthelioid fibrosarcoma (2), myxoid/round cell liposarcoma (1), malignant peripheral nerve sheath tumor (1), and other spindle, pleomorphic and undifferentiated sarcoma (9). Prior to treatment, low levels or no CD3+ T cells were seen within the tumor parenchyma; however, in virtually all of the resection specimens we observed a large number of infiltrating T cells. Increase of CD3 cells (>4 folds) were seen in 11/16 paired cases (2/18 cases had no CD3 count pre-treatment for comparison). CD4 and CD8 counts were collaborated with the CD3 count. Significant therapy effect and tumor necrosis (>50%) were seen 13/18 cases post-treatment. The tumor necrosis correlated with the increased CD3 and CD8 count.
Conclusions: Multifacted morphological and immunological assessment suggest that combination of EBRT with intratumoral injection of DC as novel neo-adjuvant treatment for STS results in significant anti-autologous tumor cell immune responses which is lacking in pre-treatment state of the host.

59 Morphologic Patterns of Residual Viable Osteosarcoma in Neoadjuvant-Chemotherapy Treated Patients: A Clinico-Pathologic Study of 101 Cases at a Single Institution

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Background: Standard treatment for osteosarcoma includes neoadjuvant chemotherapy followed by surgical resection. In treated tumors, > 90% tumor necrosis predicts favorable prognosis, necessitating accurate quantitation by the pathologist. Although criteria for residual viable osteosarcoma have been proposed, treated osteosarcomas display a variety of morphologic patterns making assessment difficult in some cases. The significance of residual cellular tumor cartilage and widely dispersed atypical cells in fibrous stroma is unknown. The purpose of this study was to address these morphologic patterns in relationship to clinical outcome.

Design: We retrospectively reviewed 101 consecutive high-grade osteosarcomas treated at our institution. The osteosarcomas subtype, chemotherapy regimen, type of resection and follow-up information were recorded. Full cross sections of the post-chemotherapy resection specimens were evaluated histologically for the presence and pattern of viable tumor cells. To calculate percent necrosis, two methods were compared: "stringent" and "inclusive." First, we counted as viable only dense areas of mitotically-active atypical cells in a peripheral, nodular or diffuse pattern ("stringent"). Second, we also included as viable rare, atypical cells in fibrous stroma and tumor cartilage ("inclusive"). Treatment response was judged as good (>90% necrosis) or poor (<90% necrosis) and correlated with recurrence-free survival using the Kaplan-Meier method.

Results: One hundred one patients (63 males, 38 females, average age 18 years (range 5- 58), average clinical follow-up 31 months (range 0 – 106)) were studied. Fifty-seven treated osteosarcomas (57%) contained nodules, peripheral rims or diffuse infiltrates of a dense population of atypical, mitotically-active, cells. Twenty-six (26 %) cases showed only widely dispersed atypical cells (n=19, 19%) in fibrosis or tumor cartilage (n=7, 7%). The remaining 18 (18%) had no evidence of residual tumor cells or cartilage. Recurrence-free survival correlated with good response in the "stringent" group (p=0.0012) but not the "inclusive" group (p=0.1855).

Conclusions: In this study, we describe the patterns of residual viable tumor commonly seen in neoadjuvant resection specimens. These data support that the percent tumor necrosis after chemotherapy is an important prognostic indicator but that areas of tumor cartilage or widely distributed, mitotically inactive, atypical cells should not be scored as residual viable tumor in the calculation.

60 Cellular Angiofibroma with Atypia and Sarcomatous Transformation

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Background: Cellular angiofibroma occurs equally in men and women and commonly arises in the inguino-scrotal or vulvovaginal regions. A prior study of 51 cases from our group showed that the tumor follows a benign course without any tendency for recurrence. In 1 prior case, an intralesional microscopic nodule of pleomorphic liposarcoma was observed. The biologic significance of atypia or sarcomatous transformation in cellular angiofibroma remains uncertain. In this study, we characterized clinicopathologic features in 11 cases of cellular angiofibroma with atypia or sarcomatous transformation.

Design: Eleven cases identified between 2001 and 2009 were retrieved from consultation files. Clinical details and follow-up were obtained from the referring pathologists. H&E-stained sections of all cases were re-examined. Immunohistochemical studies were performed on available unstained sections.

Results: Lesions arose in 11 women ranging in age from 39 to 71 years (median, 46 years). Tumor size ranged from 1.2 to 7.5 cm. In 10 cases, the tumors occurred in the vulva, and one occurred in the hip region. There were 3 cases of cellular angiofibroma with atypia. Two showed severely atypical cells and frequent mitotic figures scattered within the cellular angiofibroma. One case showed a discrete nodule of atypical cells. Eight cases of cellular angiofibroma showed sarcomatous transformation. In each case, abrupt transition to a discrete area of more pleomorphic, hypercellular tumor was seen. The sarcomatous component in 2 cases showed features of pleomorphic liposarcoma. Two cases showed a discrete nodule of atypical lipomatous tumor within cellular angiofibroma. In the remaining 4 cases, the sarcomatous component was composed of pleomorphic spindle cells. By immunohistochemistry, atypical cells and sarcomatous areas showed either multifocal or diffuse p16 expression compared to either scattered or negative expression in cellular angiofibroma. Follow-up information was available for 7 patients (range from 2 months to 75 months; median 21 months). To date, 6 patients did not develop recurrence or metastasis. One patient died of carcinoma of unknown primary.

Conclusions: Cellular angiofibroma with atypia or morphologic sarcomatous transformation occurs predominantly in the subcutaneous tissue of the vulva and, as yet, shows no evident tendency to recur based on clinical follow-up available for 7 cases. The sarcomatous component can show variable features including atypical lipomatous tumor, pleomorphic liposarcoma or pleomorphic sarcoma NOS. Overexpression of p16 suggests a possible underlying molecular mechanism.

61 "Ganglioneuroblastoma" in Adults: Potentially Aggressive Tumors

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Background: Neuroblastic tumors (neuroblastoma and ganglioneuroblastoma) in adults are extremely rare (0.3 cases per million per year according to SEER data). They reportedly have a worse prognosis than childhood cases regardless of subclassification. Despite distinct biologic differences with their pediatric counterparts, including lack of *N-myc* amplification, pediatric protocols are usually used to treat these patients. Ganglioneuromas are believed to arise from ganglioneuroblastomas that have matured. Identification of neuroblastic elements in predominantly ganglioneuromatous lesions (usually microscopic) depends upon meticulous sampling as well as uniform application of classification schemes.

Design: Cases diagnosed as ganglioneuroblastomas in adults (>18 years of age) were retrieved from 1985 – 2009 at a single institution, histologically reviewed and reclassified using INPC (International Neuroblastoma Pathology Committee) criteria. Seven cases were identified. Clinical information was tabulated as well.

Results: Patient ages ranged from 20 to 33 years (median 31 years) at presentation. All patients were female. Follow-up ranged from 0 to 6 years (median 5 months). Presenting symptoms included pelvic discomfort, pain and syncopal attacks; an asymptomatic mass was detected on pelvic exam in one patient. On histological review, 4 cases were confirmed to be ganglioneuroblastoma and 3 were reclassified as neuroblastoma. Ganglioneuroblastomas were further subclassified as nodular (1), differentiating (1) and intermixed types (2). There were metastases in 3 patients, including liver and spine (one patient) and lymph nodes (2 patients). These 3 patients expired: 1 patient 3 years after diagnosis and 2 patients after 6 years. All 4 patients with ganglioneuroblastoma and one with neuroblastoma were surgically treated. Two patients received chemotherapy. Treatment is unknown in one patient.

Conclusions: 1) Current classification schemes for neuroblastoma are inconsistently applied to adult tumors. 2) INPC criteria are probably not entirely applicable to adult neuroblastic tumors. 3) Macroscopic identification of nodular neuroblastic foci is subjective. Neuroblastic foci could be overlooked with inadequate sampling. 4) Additional clinical and pathologic review of adult neuroblastomas and ganglioneuromas could contribute to identification of risk factors in adult neuroblastic tumors and development of future treatment protocols tailored to adults.

62 Fluorescence In-Situ Hybridization for 12q15 Is a Useful Tool To Distinguish Undifferentiated Pleomorphic Sarcoma from Dedifferentiated Liposarcoma

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Background: Well differentiated liposarcomas have ring and giant marker chromosomes harboring amplification of 12q13~15 region. Fluorescence in-situ hybridization (FISH) for 12q15 amplification differentiates lipomas and atypical lipomatous tumors/well differentiated liposarcomas. We retrospectively review the clinical utility of FISH for 12q15 amplification to distinguish undifferentiated pleomorphic sarcoma (UPS) and cellular & dedifferentiated liposarcoma (DL).

Design: 16 cases ultimately diagnosed as 13 DL and 3 cellular liposarcomas were clinically evaluated with 12q15 FISH for confirmation; for comparison, 18 UPS were obtained from institutional files. In all cases, FISH was performed on unstained FFPE slides using a homebrewed 12q 15 probe and a commercial centromere 12 probe. In each case, 100 cells with ≥2 CEP12 signals were counted and amplification defined as 12q15:CEP12 ratio ≥2.5.

Results: The cellular/DLs (3 biopsies, 13 resections) involved: retroperitoneum (n=12), extremities (n=2), hip (n=1) and testicular region (n=1). All 16 cases of cellular/DLs were positive for 12q15 amplification by FISH. 8 of the 16 cases were initially considered UPS on morphologic grounds, but had 12q15 amplification demonstrated and were ultimately confirmed to be cellular or DL on with review of additional pathology slides from other cases or combined with radiologic features. The 18 UPS (3 biopsies, 15 resections) involved: extremities (n=9), retroperitoneum/pelvis/abdomen (n=6), scrotum/spermatic cord (n=2) and back (n=1). 3 of 17 (18 %) UPS cases showed 12q15 amplification. These 3 cases occurred in the abdomen, retroperitoneum and spermatic cord and could indeed represent DL with no confirming adipocytic component found.

Conclusions: Although location and radiological findings can be a useful to separate DL from UPS, these are not always sufficient. FISH for 12q15 amplification is a useful adjunct tool in distinguishing DL from UPS in certain cases lacking clear adipocytic differentiation, particularly biopsies. Lack of 12q15 amplification effectively excludes a diagnosis of DL and 12q15 amplification, while perhaps not specific, indicates that DL should be strongly considered. This test has clinical utility in distinguishing UPS from DL as the natural histories of these two neoplasms vary significantly in patterns of local/distant recurrence and outcome.

63 Impact of Molecular Analysis on the Final Diagnosis of Sarcomas: A Study on 369 Cases Collected in the Setting of a European Epidemiological Study. A Report from CONTICANET Network of Excellence

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Background: Sarcomas are rare, heterogeneous and often difficult to classify. A significant subset of sarcomas are associated with specific molecular genetic lesions such as translocations, mutations and amplifications which are helpful in the diagnosis of individual cases. However, the accurate impact of molecular genetics on the final diagnosis of sarcomas is unknown.

Design: All soft tissue and visceral sarcomas arising in patients living in 3 European areas (12 millions inhabitants) have been collected during 2 consecutive years. Every case has been reviewed by a pathologist specialised in sarcomas and a molecular analysis has been performed for any suspicion of a sarcoma with a specific genetic lesion (reciprocal translocation, mutations of KIT/PDGFRA in GIST, amplification of MDM2/CDK4 in atypical lipomatous tumor-well differentiated liposarcoma/dedifferentiated liposarcoma or ALT-WDLPS/DDLPS). In order to evaluate the impact of molecular tests, a pre-molecular analysis diagnosis has been proposed with 3 categories of

certainty: **1-**Certain diagnosis when the proposed diagnosis is the only possible one; **2-**Probable diagnosis when the proposed diagnosis is the first diagnosis contemplated, but a differential diagnostic issue is raised by other tumors**; 3-**Possible diagnosis when the proposed diagnosis is not the first diagnosis considered.
Results: During the first year, molecular analysis has been performed in 369 / 804 tumors corresponding to 166, 106 and 97 suspicion of respectively GIST, sarcomas with a translocation and ALT-WDLPS/DDLPS. Molecular analysis has been helpful (confirmation of a probable diagnosis) in 7 (4%) GIST, 34 (32%) suspicion of translocation and 31 (32%) suspicion of ALT-WDLPS/DDLPS and necessary (confirmation of a possible diagnosis) in 2 (1%) GIST, 8 (8%) suspicion of translocation and 3 (3%) suspicion of ALT-WDLPS/DDLPS.
Conclusions: In this study, the main use of molecular genetics is to confirm a suspicion of sarcoma with a translocation or an ALT-WDLPS/DDLPS in about one third of cases as well as to make possible the diagnosis of almost 10% of sarcomas with a translocation.

64 Conticabase: A Shared European Database and Virtual Tumor Bank Dedicated to Soft Tissue and Visceral Mesenchymal Tumors. A Project from CONTICANET Network of Excellence

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Background: Sarcomas are rare and heterogeneous with the need of developing joint research programmes based on common shared tumour banks. One main objective of CONTICANET, a European network of excellence with 12 clinical sarcomas Centers, is to set up a network wide, shared information system via Internet.
Design: Analysis of a questionnaire which has been circulated among partners has shown that main objectives of the database should be knowledge of tissue availability in order to initiate collaborative biologic / genomic studies and knowledge of histological, clinical, therapeutic and follow-up data in order to initiate pathological and prognostic studies; and that potential barriers could be difficulty to share data, to merge retrospective data and to collect prospective data. Users requirements were defined accordingly. The software has been developed with java and the database server is MySQL. The project has been designed according to current software engineering methodology using open source tools.
Results: Conticabase is now available (www.conticabase.org) with the following characteristics: security of the system with different rights of access, track for any access and change of data; description of data on primary tumour (clinical, pathology, treatment), follow-up, samples with paraffin embedded tissue and frozen tissue, molecular biology characteristics; possibility of import and export data from / to standard formats; query tool allowing to know the number and the list of patients corresponding to a defined category; possibility to add new parameters at the partners request; link to virtual slide repositories and genomic platforms and possibility to enter data directly from standardized reports.
Conclusions: Conticabase is a unique tool for cooperative studies and research. It is also a modern tool with link to specialised databases and it is particularly adapted to rare tumors such as sarcomas.

65 Primary Cilia Organization Orchestrating Cell Polarity in the Growth Plate and Its Loss in Osteochondroma

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Background: The growth plate is a cartilaginous template needed for the elongation of long bones. In its regulation, heparan sulphate proteoglycan (HSPG) and primary cilia play a role. Impaired HSPG biosynthesis is associated with osteochondroma formation. Primary cilia function as cell's antennas that receive and transduce mechanical and chemical signals from the surrounding cells and the extracellular matrix.
Design: We evaluated the organization of primary cilia in the growth plate (n=5) and osteochondroma (n=5) and its relation with cell polarity. The cilium was immunolabed with acetylated α-tubulin antibody and its organization was studied by confocal microscopy. The centrioles were stained with γ-tubulin. The ciliary assembly was analyzed by electron microscopy and by the immunolocalization of KIF3A motor protein.
Results: The primary cilia organization in the growth plate reflected that chondrocytes non-polarized (resting chondrocytes) became polarized (proliferating and hypertrophic chondrocytes) orienting the cilium parallel to the longitudinal axis of the bone. The primary cilia alignment formed one virtual axis which crossed the center of column of chondrocytes. The centrioles organization also confirmed the presence of the virtual axis. The ciliary axes showed the polarity axis of the growth plate. In osteochondroma, primary cilia were randomly located in the central or in the lateral-medial region of the cells related to the growth axis of the tumor. Interestingly, the virtual axis was also found in the osteochondroma cells focally arranged into column. The primary cilia organization in osteochondroma reflected loss of cell polarity. The ciliary assembly, as judged by electron microscopy and the immunolocalization of KIF3A motor protein, was similar in both. We also demonstrated the dynamicity of primary cilia, whose presence/absence was correlated with the cell cycle.
Conclusions: We showed that the organization of primary cilia in the growth plate reflects cell polarity. The achievement and maintenance of individual and collective polarity in the growth plate is essential for its normal regulation. The loss of cell polarity in osteochondroma seems to be a key event and might be related with its formation and indicate abnormal transduction of signaling molecules or impaired cell-matrix interaction.

66 P63 Is Not a Helpful Marker in the Diagnosis of Giant Cell Tumor of Bone

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Background: Giant cell tumor of bone (GCT) is a locally aggressive neoplasm with high recurrence rate that occurs mainly in long bones of young adults. While the diagnosis is often straightforward, it can be challenging with small core needle biopsies, particularly when dealing with unusual sites or skeletally immature patients. The differential diagnosis includes both benign and malignant neoplasms of bone and soft tissue that are rich in giant cells. Recent studies using immunohistochemistry and molecular methods on tissue microarray samples have demonstrated overexpression of p63 in the stromal cells of most giant GCTs and advocate the use of p63 as diagnostic marker of GCT.
Design: 87 cases of giant cell lesions of bone and soft tissues, including 23 giant cell tumors of bone, 8 primary aneurysmal bone cysts (ABC), 15 tenosynovial giant cell tumors (TSGCT), 12 chondroblastomas (CHB), 4 giant cell reparative granulomas (GCRG), 6 non-ossifying fibromas (NOF), 2 fibrous dysplasias (FD), 4 pigmented villonodular synovitis (PVNS), and 4 osteosarcomas with giant cells (OS), were selected from our pathology files. Only cases with abundance of giant cells were selected for the study. Except for one needle biopsy, all cases were resection samples, open biopsies, or bone curettages. All routine histologic sections were reviewed and one or two paraffin blocks were selected for p63 immunohistochemistry. Immunoreactivity was scored by intensity of nuclear staining as weak (1+), moderate (2+), and strong (3+) and also by percentage of staining cells as focal (<10%), intermediate (10-50%), and diffuse (>50%).
Results: p63 reactivity was identified in 20 of 23 GCTs (86.9%), 5 of 8 primary ABCs (62.5%), 10 of 12 CHBs (83.3%), 4 of 4 GCRGs (100%), 2 of 4 OS (50%), 1 of 15 TSGCT (6.6%), 1 of 6 NOF (16.6%), and 1 of PVNS (25%). The immunoreactivity was only seen in stromal cells. While the intensity and percentage of cells staining was variable, strong and moderate staining was seen in most GCTs and some non-GCT cases. In CHBs, significant reactivity was seen in chondroid areas. The sensitivity, specificity, positive predictive value and negative predictive value of p63 immunohistochemistry for the diagnosis of GCT was 86.95%, 62.50%, 45.45%, and 93.02%, respectively.
Conclusions: These findings indicate that p63 immunohistochemistry is not a helpful diagnostic marker in the diagnosis of GCT since a significant number of other giant cell lesions express this marker.

67 Evaluation of Concordance between Initial Diagnosis and Central Pathology Review in a Comprehensive Series of Sarcoma Patients Diagnosed in 3 European Regions

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Background: Sarcomas are tumors difficult to diagnose accurately. Over a one-year period, we have reviewed all sarcomas diagnosed within three European regions (Rhone-Alpes (RA) and Aquitaine (Aq) in France, Veneto (Ve) in Italy, representing 12 millions inhabitants). The goal of this work was to assess the similarity between initial diagnosis and expert review.
Design: All primary soft tissue and visceral sarcomas were separated into two main groups: cases sent for the systematic review (SR) induced by this study and cases sent spontaneously for second opinion (SO). Within each group, the initial diagnosis was compared with the corresponding expert opinion. The similarity of the two diagnoses was assessed according to 3 defined levels: (0) absolute mismatch: major difference between the two diagnoses e.g. sarcoma vs benign soft tissue tumor or non-sarcoma, or misidentification of the subtype of sarcoma potentially resulting in important therapeutic impact; (1) partial concordance: inconsistence with the grade or the sarcoma subtype without therapeutic consequence, and (2) absolute concordance: identical diagnoses.
Results: 765 cases were analyzed and corresponded to: 347 patients (45%) from RA, 172 patients (23%) from Aq, and 246 (32%) from Ve. SR represented 60% of the cases (RA=170/347, 49%; Aq=75/172, 44%; Ve=215/246, 87%) and SO the remaining 40% (RA=177/347, 51%; Aq=97/172, 56%; Ve=31/246, 13%). Within the SR group, the three types of concordance rates observed were: 7% (RA=10/170, 6%; Aq=6/75, 8%; Ve=14/215, 7%) of total discordance, 27% (RA=48/170, 28%; Aq=25/75, 33%; Ve=50/215, 23%) of partial concordance, and 66% (RA=112/170, 66%; Aq=44/75, 59%; Ve=151/215, 70%) of total concordance. Within the SO group, the first diagnosis assumption of the pathologist was arbitrarily set as the initial diagnosis to be compared with the expert SO. The total discordance rate reached 18% in this group (54/305).
Conclusions: The high rate of spontaneous SO (40%) shows that pathologists are aware of the difficulties they face to make reliable diagnosis in this complex pathology. The 7% total discordance rate within a group of patients that would not have benefited from any review without this study (SR), shows the necessity to extend the expert process.

68 Reproducibility of Molecular Analysis in Soft Tissue Sarcomas and GIST: A Report from CONTICANET Network of Excellence

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Background: Molecular analysis and molecular genetics is being gradually implemented in the diagnostic workout of soft tissue sarcomas and GIST. Data regarding reproducibility as well as variability of molecular testing are lacking. A study has been performed within the Conticanet European Network of Excellence in order to evaluate the degree of concordance of molecular testing across three european referral laboratories.

Design: All soft tissue and visceral sarcomas (804 cases) occurring in 3 european regions (Veneto Italy, Aquitaine and Rhone-Alpes, France) have been collected prospectively during 2007. All cases of GIST and all cases of sarcoma in which a translocation was suspected were analyzed by FISH and RT qPCR. 10 GIST and one to every 10 sarcomas have been circulated among the three partners 44 cases (25 GIST and 19 sarcomas with a suspicion of translocation) were molecularly analysed. In 43 additional cases the results of FISH versus RT qPCR were compared. In 21 cases molecular tests were s also performed and compared on both frozen and FFPE material.
Results: Among GIST, 96% concordance was observed (one exon 18 PDGFRA mutation not detected in one region). Among sarcomas (10 synovial sarcomas, 4 myxoid liposarcomas, 2 rhabdomyosarcomas, 2 clear cell sarcomas, and 1 desmoplastic tumour) 89% concordance was observed. Discordances were in 2 myxoid liposarcoma (DDIT3/FUS translocation detected in two regions not confirmed in one region). 43 cases were evaluated for translocations by both RT-qPCR and FISH: 15 synovial sarcomas, 12 myxoid liposarcoma, 9 low grade fibromyxoid sarcoma, 4 Ewing's sarcoma/PNET and 3 alveolar rhabdomyosarcoma. Only one discordance was observed (one negative RT-PCR/positive FISH myxoid liposarcoma) however, 2 cases were not interpretable by RT-PCR as compared to 7 cases by FISH. Non discrepancies were observed between frozen and FFPE specimens.
Conclusions: The level of concordance of molecular testing among the three referral centers is remarkably high. The discrepancy observed in myxoid liposarcoma may be due to higher technical complexity. RT-PCR appears more sensitive than FISH in detecting sarcoma translocations. Molecular analysis performs optimally in both frozen and FFPE material.

69 Benign Notochordal Cell Proliferations in the Ferret: An Animal with a High Incidence of Chordoma

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Background: Chordoma is an uncommon malignant tumor of the axial skeleton. Chordoma rarely affects other animals except for ferrets, in which it is a well-recognized neoplasm that develops in the tip of the tail. In humans, chordoma is hypothesized to arise from benign notochordal cell tumors (BNCT) that have been identified in the vertebral column of up to 20% of humans, primarily within the sacral and occipital regions. To further understand the relationship between benign notochordal lesions and chordoma and to identify a relevant animal model, we examined the vertebrae of the tails of a group of ferrets.
Design: Tails from 6-8 month old wild-type research ferrets were fixed in formalin, decalcified, and bisected longitudinally. All of the vertebrae were sectioned, processed routinely, and examined histologically for the presence of benign notochordal proliferations (BNP). When identified, the lesional tissue was stained immunohistochemically with keratin.
Results: Thirty-two ferret tails were examined. All of the intervertebral discs contained centrally located nucleus pulposus composed of notochordal cells. The vertebral bodies contained cancellous bone and hematopoietic marrow. Two benign notochordal proliferations (BNP) were identified, one of which was confirmed by a keratin stain. One BNP was located in the shaft of the tail (larger), whereas the other (smaller) arose in the most distal tail vertebrae. Histologically, the larger lesion was associated with thickened bony trabeculae and replaced portions of the marrow. The BNP were composed of sheets of polyhedral cells with eosinophilic or clear vacuolated cytoplasm. There was no discernable myxoid stroma. No mitoses were identified. The lesional cells stained strongly with keratin.
Conclusions: Benign notochordal cell proliferations occur in ferrets, an animal that is recognized to have a relatively high incidence of chordoma. This finding suggests that ferrets may be an appropriate animal model to study the mechanisms driving benign and malignant notochordal proliferations.

70 Phosphorylated Ezrin Is Located in the Nucleus of the Osteosarcoma Cell In Vitro and In Vivo

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Background: The survival of osteosarcoma patients is connected to metastasis. The ezrin expression was associated with the development of metastasis and poor outcome in osteosarcoma. Ezrin is present in the cytoplasm and after phosphorylation assumes an active form and links F-actin to the cell membrane. The study evaluated ezrin and phosphorylated ezrin (p-ezrinTyr354 and p-ezrinThr567) expression and its sub-cellular localization in osteosarcoma.
Design: We studied 50 osteosarcoma patients (mean follow-up 9,8 yrs). Ezrin expression were assessed using immunohistochemistry (IHC) and immunofluorescence (IF) analysis on tissue microarray (TMA) and cultured cells of human osteosarcoma (143B). The Western Blot (WB) analysis was performed on 143B.
Results: The majority of osteosarcomas, shown cytoplasmic positivity for ezrin, phosphorylated and un-phosphorylated, were associated with membranous and nuclear positivity for p-ezrinThr567 and p-ezrinTyr354 respectively. The IF and WB analysis confirmed the nuclear localization of p-ezrinTyr354. Ezrin expression was associated with high grade osteosarcoma (p=0,04), with metastasis (p=0,04) and with tumors that developed metastasis (p=0,04); p-ezrinThr567 expression was present mostly in tumors with respect to metastasis (p=0,01) and in osteosarcomas that didn't develop metastasis (p=0,002). The osteosarcoma patients with ezrin expression have a short survival.
Conclusions: The cytoplasmic ezrin expression matches with its role of membrane-cytoskeleton linker protein, also in osteosarcoma. The p-ezrinTyr354 nuclear localization suggests its possible role as a nuclear factor in osteosarcoma. The ezrinThr567 phosphorylation may not be necessary in osteosarcoma metastatic progression but it was modulated. The ezrin expression is associated with a more aggressive osteosarcomas and with the metastatic process.

71 Adamantinoma and Osteofibrous Dysplasia: Diagnostic Application of p63 with Additional Evidence for a Pathophysiologic Relationship

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Background: Adamantinoma is a low-grade primary bone neoplasm demonstrating epithelial differentiation. It exhibits some degree of clinical overlap with osteofibrous dysplasia; indeed, a relationship between these lesions has previously been proposed on the basis of cytogenetic similarities and co-expression of keratin. We report the presence of p63 expression in the epithelial component of adamantinomas and sought to determine whether this putative stem cell marker, which is also associated with epithelial-mesenchymal interactions, was also expressed in osteofibrous dysplasia.
Design: We performed a retrospective review of our archives to identify cases of adamantinoma and osteofibrous dysplasia. Five cases of fibrous dysplasia served as controls. H&E stained sections of paraffin embedded tissue were reviewed by light microscopy to confirm the diagnosis. Sections from each case were subsequently stained for p63 using standard immunohistochemical methods.
Results: Nine cases of adamantinoma (six classical, three osteofibrous dysplasia-like) and ten cases of osteofibrous dysplasia were identified. The epithelial component in all cases of adamantinoma was found to express p63. The percentage of epithelial cells expressing this marker appeared greatest in the classical variant. Despite the absence of an epithelial component in osteofibrous dysplasia, rare scattered cells expressing p63 could be identified in eight cases of osteofibrous dysplasia. These cells were scattered amongst the spindle cells and did not appear to be associated with either the vasculature or bone trabeculae. None of five cases of fibrous dysplasia were found to contain p63 expression.
Conclusions: To our knowledge this is the first report of p63 expression in the epithelial cell component of adamantinoma. Moreover, the presence of staining, albeit focal, in osteofibrous dysplasia supports the notion of a relationship between osteofibrous dysplasia and adamantinoma. It remains unclear whether p63 is highlighting a stem cell within osteofibrous dysplasia – a possibility that warrants further consideration.

72 Meta-Mining of Gene Expression Profiles Identifies MG132 and Cantharidin as Therapeutic Agents with In Vitro Activity Against Leiomyosarcoma

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Background: Few therapeutic agents exist for the treatment of leiomyosarcoma (LMS). In a prior study, we performed molecular profiling of LMS and identified 3 LMS subtypes. In the present study, we used the gene expression signatures of the LMS subtypes to identify novel therapeutic compounds for the treatment of LMS, and we used in vitro experiments to test their efficacy.
Design: Lists of the top 200 differentially expressed genes in each of the 3 LMS subtypes (n=49) compared with a set of leiomyomas (n=19) was uploaded into the Connectivity Map (CMap, www.broadinstitute.org/cmap), a resource designed to find connections between gene expression profiles and drug response signatures. We selected 4 highly ranked candidate therapeutic compounds with strong negative or positive enrichment with at least 1 of the LMS subtypes. We tested the effectiveness of the compounds against the 3 LMS cell lines. Experiments were carried out in quadruplicate using 2-fold serial dilutions of each drug. Viability was assessed using the XTT Cell Proliferation Kit II (Roche Diagnostics, Mannheim, Germany).
Results: Expression profiling demonstrated that the 3 LMS cell lines each showed the most similarity with LMS subtype II. Cantharidin, a protein phosphatase inhibitor, showed the strongest negative enrichment across all 3 subtypes in the CMap analysis and was effective in inhibiting the growth of the 3 LMS cell lines. Tyrophostin AG-825, an inhibitor of the HER-2/neu tyrosine kinase, showed a negative enrichment with LMS subtypes I and II had no significant effect on cell viability in the 3 LMS cell lines. MG132, a proteasome inhibitor with a strong positive enrichment with LMS subtype II, showed a strong negative effect on cell viability in the 3 LMS cell lines. Doxorubicin, the most commonly used chemotherapeutic treatment for LMS, showed moderately negative enrichment with LMS subtype II and showed a moderate, though non-uniform, inhibitory effect against the 3 LMS cell lines.
Conclusions: The CMap analysis revealed novel candidate drugs for the treatment of LMS. The drug sensitivity experiments demonstrated that MG132 and Cantharidin show activity against LMS cell lines. These experiments provide a starting point for further mechanistic studies of LMS drug sensitivities in vitro and in vivo and represent a first step towards the development of personalized therapies for LMS patients.

73 Mammalian Target of Rapamycin (mTOR) Is Constitutively Activated in Chordomas

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Background: Chordomas are rare neuroaxial tumors that are locally aggressive and notoriously resistant to chemotherapy and radiation therapy. Recent data suggest that mammalian target of rapamycin pathway could play a role in the oncogenesis of chordomas and thus an important therapeutic target. Mammalian target of rapamycin (mTOR) is a serine/threonine kinase of the PI3K/AKT signaling pathway known to play an important role in cell growth and has been shown to be activated in various solid organ tumors and sarcomas. Several mTOR inhibitors are currently in clinical

trials. However, expression of mTOR pathway-related genes in chordomas have not been completely evaluated on routine histologic material by immunohistochemical staining for phosphoribosomal S6 protein (p-s6rp), phospho-mTOR, p70S6K and p-AKT antibodies.

Design: 16 cases of chordoma were retrieved from the files at the Hospital of the University of Pennsylvania and the Ohio State University. Representative section from each case were stained with antibodies to p-AKT, p-mTOR, p-70S6k and p-S6rp (Ser235/236) using the DAKO autostainer. Staining was evaluated semiquantatively for both quantity (%: 0,≤10%, ≤25%, ≤50%, >50%) and intensity (0,1+ to 3+). Negative staining was defined as staining of 0 and 1+ <5%.

Results: In all the cases, the tumor cells showed intermediate to intense staining (2+ & 3+; 100%) with p-mTOR and p-70S6k antibodies; 14 of the 16 cases showed 2+ or 3+ p-aKT staining. P-S6rp staining was positive (2+ & 3+) in 14 of the 16 cases. The results are summarized in the table below:

pAKT/p-mTOR/p-70S6K/p-S6rp Expression in Chordomas

Stains	p-AKT	p-mTOR	p-70S6K	p-S6rp
Chordomas (n=16) % staining	94	100	100	89

Conclusions: The results of a high level of expression for the p-AKT, p-mTOR, p-70S6k and pS6rp antibodies, are indicative of a high frequency of constitutive activation of PI3K/AKT/mTOR pathway in chordomas as previous suggested. Our study supports the use of mTOR inbihitors in treatment of chordoma and needs to be further evaluated in experimental models and clinical trials.

74 Mammalian Target of Rapamycin (mTOR) Pathway Is Differentially Upregulated in Liposarcomas More Than Lipomas and Normal Fat

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Background: The mammalian target of rapamycin (mTOR) is a protein kinase of the phosphatidylinositol 3-kinase (PI3K) signaling pathway that regulates cell growth and survival. The mTOR pathway is activated in various solid organ tumors and sarcomas, and is of importance because several mTOR inhibitors are currently on clinical trials. Phosphoribosomal S6 protein (p-S6rp) is a surrogate marker for mTOR pathway activation and has been shown to be predictive of tumor response to mTOR inhibition. This is the first study on the differential activation of the mTOR pathway in normal fat, lipomas and liposarcomas.

Design: 38 lipomatous lesions including normal fat (n=10), lipomas (n=10), and liposarcomas (n=18), including well differentiated liposarcoma, (n=10), myxoid/round cell liposarcoma (n=6) and pleomorphic liposarcoma (n=2) were retrieved form the case files of OSU. Standard sections were stained with anti-pS6rp (Ser 235/236, Cell Signaling Technologies, Danvers, MA, USA) using the DAKO autostainer. Staining was evaluated semiquantatively for both quantity (%: 0,≤10%, ≤25%, ≤50%, >50%) and intensity (1-3+). Negative staining was defined as staining of 0 or 1+ intensity of <5%.

Results: A high level of expression of p-s6rp was observed across the various subtypes of liposarcomas (LPS) with (89%; 16/18) 2-3+ staining intensity. The well differentiated LPS's had a positive rate of 80% (8/10 cases) while all cases of myxoid/round cell LPS (n=6) and pleomorphic LPS (n=2) were positive (100%) respectively. Lipomas showed an intermediate level of expression (60%), with the majority of cases showing 2+ expression in approximately 10% of tumor cells. Normal fat had a positive rate of 30%. The differential staining between the LPS's, lipomas and normal fat on chi-square analysis was statistically significant with p≤0.05≤0.001 respectively.

Phospho-S6 ribosomal protein expression in Liposarcomas, Lipomas and Normal Fat

Tumor types	Positive	Negative	Total
Liposarcomas (n=18)	16	2	18
Lipomas (n=10)	6	4	10
Normal Fat (n=10)	3	7	10

Conclusions: The relative high expression of p-S6rp in malignant fat tumors compared to lipomas and normal fat is indicative of the differential activation of the mTOR pathway in liposarcomas with potential implication for the use of mTOR inhibitors for targeted therapy. Clinical trials are needed to validate the utility of these novel agents.

75 Quantitative Analysis of Carboxypeptidase M mRNA Expression in Lipomas and Liposarcomas

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Background: Well-differentiated liposarcomas/atypical lipomatous tumors (WDL/ALT) are cytogenetically characterized by ring and giant rod chromosomes. These abnormal chromosomes contain several amplified genes including *MDM2*, *CPM*, *CDK4, TSPAN31*, and others. As part of a large genomic study of lipomatous neoplasms, we initially found Carboxypeptidase M (*CPM)* to be consistently amplified in well-differentiated liposarcoma/atypical lipomatous tumors but not in lipomas (Modern Pathol 2009). In this study we evaluated and compared *CPM* mRNA expression levels in these groups of tumors.

Design: Relative quantification of *MDM2* and *CPM* were performed by quantitative real-time PCR (qPCR) in 9 WDL/ALT, 3 dedifferentiated (DD) liposarcomas, 1 pleomorphic sarcoma, 2 lipomas, and 2 normal adipose tissues. All analyses were performed in triplicate using UPL probes and a Light Cycler® 480 System (Roche, Mannheim *Germany*). Based on PCR efficiencies for both *CPM* and *MDM2*, using LC 480 1.5 software, relative differences between genes were determined using a standard curve generated from samples with perfect PCR efficiency. Concentrations of each sample were calculated from Cp values, and concentration of the target genes were divided by the concentration of the reference gene (*PGK*), thus providing the ratio. Fold induction of transcription of *MDM2* and *CPM* were estimated by comparing to the values of *PGK*. Comparative and correlative expression analyses were performed using non-parametric methods.

Results: qRT-PCR showed that *CPM* mRNA expression levels were higher in WDL/ALT and DD liposarcomas (*CPM* medians 5.55 and 6.41, respectively) than in lipomas or normal fat (*CPM* medians 1.26 and 2.11, respectively) (p=0.005). Similar results were found with *MDM2* mRNA for WDL/ALT and DD liposarcomas (*MDM2* medians 8.32 and 24.47, respectively) and lipomas or normal fat (*MDM2* medians 1.23 and 1.16, respectively) (p=0.001). *CPM* and *MDM2* mRNA expression levels in pleomorphic liposarcoma were low (1.01 and 0.11, respectively). *CPM* mRNA expression levels were moderately correlated with *MDM2* mRNA expression (r=0.61; p=0.007).

Conclusions: Similar to *MDM2*, *CPM* is transcriptionally upregulated in WDL/ALT and DD liposarcoma but not in lipomas, normal adipose tissue, and in a single example of pleomorphic liposarcoma. These results suggest a functional role of *CPM* in the biology of WDL/ALT and DD liposarcoma and further validate its value as a novel biomarker for the diagnosis of these tumors.

76 Low-Grade Fibromyxoid Sarcoma. A Clinicopathologic Study

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Background: Previous studies of low-grade fibromyxoid sarcoma have had too few cases, too little follow-up, or both to provide a comprehensive pathologic and clinical picture of the tumor.

Design: Cases of low-grade fibromyxoid sarcoma in our files were reviewed. They were included if there was adequate histologic material and clinical information with at least 5 years follow-up.

Results: Thirty-three cases met the study criteria. The patients were 6 to 52 years old (median 29 years); 19 were male and 14 female. The most common tumor locations were the shoulder area (5), thigh (5), and inguinal area (4). Tumor size varied from 1.5 cm to 16 cm (median 9.4 cm) where it was known. The typical histologic findings were contrasting fibrous and myxoid areas, moderate to low cellularity, bland-appearing spindle cells with no or slight nuclear pleomorphism and rare mitotic figures, and a swirling, whorled growth pattern. Prominent vascularity in myxoid areas and perivascular hypercellularity were fairly common, whereas larger hypercellular zones were sometimes seen in primary tumors but were more frequent in recurrences (local) and metastases. Hypercellular regions sometimes had round rather than spindle cells and a somewhat increased mitotic rate. Features less often observed included focal moderate nuclear pleomorphism (usually in recurrences), areas with a storiform, straight, fascicular or diffuse cell arrangement, thick collagen bundles, and osseous metaplasia. Pericollagenous rosettes were present in 6 cases but not in all specimens from these. One patient had a recurrence with features of sclerosing epithelioid fibrosarcoma, whereas 2 had dedifferentiated recurrences with anaplastic predominantly round cells and numerous mitotic figures. Thirteen patients died of tumor after 3 (tumor became dedifferentiated) to 31½ years (median 11 years). Twenty patients were alive at latest follow-up of 5 to 67 years (median 12 years), 6 with tumor and 14 without. Twenty-one patients had recurrence after intervals of up to 15 years (median 3½ years), and 15 had metastases (mostly in lungs and pleura) after periods of up to 45 years (median 5 years). Except for dedifferentiation, which led to short survival after it occurred, histologic differences were not related to tumor behavior or patient survival. The 4 patients whose neoplasms measured less than 3.5 cm were all tumor-free at latest follow-up.

Conclusions: Low-grade fibromyxoid sarcoma has a range of histologic features beyond the typical pattern, but these differences do not relate to tumor behavior or patient survival. Small tumor size may be a favorable prognostic factor.

77 Immunohistochemical Staining for TLE1 Distinguishes Synovial Sarcoma from Histologic Mimics

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Background: TLE1, a transcriptional corepressor implicated in hematopoiesis and epithelial differentiation, is overexpressed in synovial sarcomas. Recent studies evaluating its utility as a diagnostic marker have reported conflicting results. We investigated TLE1 expression by immunohistochemistry in a well-characterized series of synovial sarcomas and other mesenchymal tumors most commonly considered in the differential diagnosis.

Design: Whole tissue sections of 171 tumors were evaluated: 54 synovial sarcomas (21 biphasic, 18 monophasic, 15 poorly differentiated), 40 malignant peripheral nerve sheath tumors (MPNST; all positive for S100 and/or arising in patients with type 1 neurofibromatosis), 40 solitary fibrous tumors (20 typical and 20 histologically malignant, defined by mitotic activity), 20 fibrosarcomatous variant of dermatofibrosarcoma protuberans, and 17 Ewing sarcoma/primitive neuroectodermal tumors (PNET). All monophasic and poorly differentiated synovial sarcoma and Ewing sarcoma/PNET cases were previously confirmed to harbor t(X;18) or *EWSR1* gene rearrangement, respectively. Immunohistochemical studies were performed following pressure cooker antigen retrieval using a polyclonal anti-TLE1 antibody (1:400; Santa Cruz). The extent of immunoreactivity was graded according to the percentage of tumor cells showing nuclear staining: 0, no staining; 1+, <5%; 2+, 5-25%; 3+, 26-50%; 4+, 51-75%; or 5+, 76-100%; and the intensity of staining was graded as weak, moderate, or strong.

Results: In total, 41 of 54 (76%) synovial sarcoma cases showed TLE1 positivity, including 16 (76%) biphasic (5+ [4], 4+ [2], 3+ [3], 2+ [3], 1+ [4]; weak [9], moderate [6], strong [1]), 12 (67%) monophasic (5+ [4], 4+ [3], 3+ [0], 2+ [5], 1+ [0]; weak [5], moderate [5], strong [2]), and 13 (87%) poorly differentiated (5+ [6], 4+ [2], 3+ [1], 2+ [3], 1+ [1]; weak [1], moderate [6], strong [6]). Of the other tumor types evaluated, only 4 (10%) MPNSTs were positive for TLE1 (3+ [1], 2+ [2], 1+ [1]; weak [2], moderate [2]). All of the other tumors were completely negative for TLE1.

Conclusions: TLE1 is a sensitive and highly specific marker for synovial sarcoma and can be helpful to distinguish synovial sarcoma from histologic mimics. In this study, only a small subset of MPNST showed limited staining for TLE1.

78 Aneurysmal Bone Cyst with Dual Rearrangements of SS18 and USP6

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Background: Aneurysmal bone cyst (ABC) is a benign osteolytic lesion containing hemorrhagic cystic regions surrounded by a heterogeneous mixture of mesenchymal cells. Rearrangements of the USP6 (also known as TRE2 or TRE17) oncogene are present in approximately 70% of lesions classified as aneurysmal bone cyst. We report a case of a 30 year old woman with a right scapular bone cyst, with classic histologic features of an aneurysmal bone cyst and simultaneous rearrangements of the SS18 (also known as SYT) and USP6 gene loci.

Design: Biopsy tissue was submitted to the pathology laboratory for histologic evaluation, and a portion of fresh tumor was submitted for cytogenetics. RT-PCR for the SS18-SSX1 and SS18-SSX2 translocations was performed on FFPE tissue. FISH analysis for SYT (SS18) was performed on cultures and FFPE sections. FISH analysis for USP6 was performed on FFPE sections using selected bacterial artificial chromosome (BAC) clones flanking the USP6 gene. BAC clones were labeled with Spectrum Green (telomeric) and Spectrum Orange (centromeric) and probe localization was confirmed on normal peripheral blood metaphase cells prior to analysis by fluorescence microscopy.

Results: The resected lesion had classic histologic features of aneurysmal bone cyst. Cytogenetic analysis was performed and showed a complex karyotype: 45,t(X;18;4)(p11.2;q11.2;q13),del(8)(p21),-13,add(17)(p11.2) in twenty cells analyzed. A rearrangement of SS18 was observed in 78% of interphase cells by fluorescence in situ hybridization (FISH) on cultured material. RT-PCR for the SS18-SSX1 and SS18-SSX2 translocations showed no evidence of the X;18 translocation associated with synovial sarcoma. FISH analysis on areas of tumor from FFPE tissue sections showed a rearrangement of SS18 in 25% of nuclei and a rearrangement of USP6 in 24% of nuclei.

Conclusions: We describe a dual rearrangement of SS18 and USP6 in a case of aneurysmal bone cyst with a complex karyotype. Rearrangement of USP6 by FISH in a similar percentage of cells (24%) compared to cells with SS18 rearrangement (25%) suggests a cryptic chromosomal rearrangement with a fusion of SS18 (18q11.2) to USP6 (17p13). This novel rearrangement probably places the coding region of USP6 under the control of SS18 promoter sequences. This case shows that rearrangement of the SS18 (SYT) locus is not conclusive evidence of synovial sarcoma, and may be present in tumors other than synovial sarcoma.

79 Diagnostic Accuracy of Core Needle Biopsy of Bone Lesions: Analysis of 230 Cases

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Background: Core needle biopsy has many advantages in the treatment of bone tumors. The accurate diagnosis of bone tumors is important for successful treatment. The aim of this study is to assess the diagnostic value of fluoroscopy-guided core needle biopsy in the bone lesions.

Design: Between 2004-2009, 230 bone biopsies were performed by Jamshidi needle. The diagnoses of these biopsies were compared with final diagnoses of open biopsy and/or definitive operation.

Results: One hundred eighty eight specimens (81.7%) were determined to be adequate for histologic diagnosis. An experienced pathologist on bone pathology evaluated all cases. One hundred and seven cases were evaluated as benign, 81 cases were malign. Osteosarcoma and giant cell tumor were the most common tumors of malignant and benign cases respectively. In our series the overall sensitivity was 98.4% and specifity was 96.2%. There were one false-positive and three false-negative diagnoses. The positive predictive value of core needle biopsy was 99.4 %.

Conclusions: The results indicate that needle biopsy in bone lesions is safe, easy to perform, requires small amount of tissue and permit high diagnostic accuracy.

80 Diagnostic Application of Tumor Type-Specific Fusion Genes to Surgical Pathology of Bone and Soft Tissue Tumors: Experience Based on 200 Consultation Cases

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Background: Molecular detection of tumor type-specific fusion genes is a powerful diagnostic tool of bone and soft tissue tumors, which often create diagnostic challenges in surgical pathology. To highlight its utility and limitation, we itemize the consultation cases in which the molecular genetic testing was applied to their pathologic diagnoses.

Design: Among more than 4000 consultation cases sent to us in 1999-2008, we selected 200 consecutive cases in which RT-PCR-based molecular detection of a variety of fusion genes was performed using formalin-fixed, paraffin-embedded tissues (FFPE). Clinicopathologic findings of each case were reviewed and assessed with their initially assumed and final diagnoses.

Results: In the consultation cases, synovial sarcoma (39%) and the Ewing family of tumors (23%) were the most frequently intended tumor types for the molecular genetic testing. In 196 cases with informative RNA extracted from FFPE, tumor type-specific fusion gene transcripts were detected in 95 cases including six (three synovial sarcomas, one desmoplastic small round cell tumor, one extraskeletal myxoid chondrosarcoma and one dermatofibrosarcoma protuberans) morphologically and 33 clinically (i.e. age and anatomical location) unusual tumors. Although 72 of the 101 cases without detectable fusion gene transcripts showed more or less unusual histologic features as the tumor types amenable to their specific PCR, the other 21 cases were morphologically typical or compatible with translocation-associated sarcomas such as Ewing tumor (48% of 21 cases) and synovial sarcoma (29%). With the application of a novel primer set to the PCR, a rare splicing variant of SYT-SSX1 was detected in one such synovial sarcoma case that was initially 'fusion-negative'. No histologic slides were available for reviewing in the remaining eight cases lacking detectable fusion transcripts.

Conclusions: Our data suggests that the RT-PCR-based detection of the tumor type-specific fusion genes using FFPE is a robust molecular technique for confirming the diagnoses of translocation-associated sarcomas particularly in cases with unusual clinicopathologic features. In 'fusion-negative' but morphologically consistent cases, some technical modifications may improve detection sensitivity. In addition, an extended molecular testing would expand the morphologic diversity of translocation-associated sarcomas.

81 Round Cell Inflammatory Myofibroblastic Tumor with Nuclear Membrane or Perinuclear ALK: An Aggressive Intra-Abdominal Variant

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Background: Inflammatory myofibroblastic tumor (IMT) is a mesenchymal neoplasm of intermediate biologic potential that may recur and rarely metastasize. Pathologic features do not correlate well with behavior. Approximately 50% of IMTs harbor ALK gene rearrangement and overexpress ALK, most demonstrating diffuse cytoplasmic staining. Rare IMTs with a distinct nuclear membrane or perinuclear pattern of ALK and round cell morphology have been reported. These cases showed an aggressive clinical course, suggesting that such patterns may predict malignant behavior.

Design: IMTs from 4 institutions were screened to identify tumors showing nuclear membrane or perinuclear ALK. Clinicopathologic features were evaluated, and follow-up was obtained. FISH and RT-PCR were performed on a subset of cases.

Results: 8 cases were identified. All patients were male (mean age 35 yr; range 7 mo – 59 yr), and all tumors were intra-abdominal (mesentery or omentum). Mean size was 16 cm (range 8–26 cm); 3 were multifocal. The tumors were composed predominantly of sheets of round to epithelioid cells with vesicular nuclei, large nucleoli, and amphophilic cytoplasm. In all cases, a minor spindle cell component was present; 5 had abundant myxoid stroma. In 4 cases neutrophils were prominent, in 3 cases lymphocytes. Median mitotic rate was 6 per 10 HPF; 5 had necrosis. All were positive for ALK (6 nuclear membrane pattern; 2 perinuclear), 7 desmin, 3 focal SMA, and 5 focal CD30. All were negative for myf4, keratins, EMA, and S-100. Thus far, FISH was positive for ALK rearrangement in 2 cases, and in 1 case a RANBP2-ALK fusion was detected by RT-PCR. Follow-up ranged from 3 mo - 3 yr (median 13 mo). Seven patients underwent surgical resection; in 1 case, tumor was inoperable. All patients had rapid local recurrence (within 8 mo); 4 had multiple recurrences. Seven had post-operative chemotherapy; 2 had XRT. Two patients also developed metastases (1 liver; 1 lung). Thus far, 3 patients died of disease, 4 are alive with disease, and 1 has no evidence of disease.

Conclusions: Round cell IMT with nuclear membrane or perinuclear ALK is a distinctive variant with an intra-abdominal predilection. Unlike conventional IMT, abundant myxoid stroma and prominent neutrophils are common. These tumors pursue an aggressive course with rapid local recurrences.

82 Low Level MDM2 Gene Amplification and MDM2 Polysomy Are Found in Extra-Uterine and Uterine Leiomyosarcoma by Fluorescence In Situ Hybridization (FISH) Analysis

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Background: Assessment of MDM2 gene copy number by fluorescence in situ hybridization (FISH) analysis is of particular diagnostic utility in the setting of well-differentiated and de-differentiated liposarcoma, which exhibit MDM2 amplification, and in pleomorphic lipoma, which exhibits MDM2 polysomy. Although many non-lipomatous mesenchymal tumors have been shown to lack MDM2 alterations, leiomyosarcomas have not been well-characterized with regard to MDM2. The purpose of this study was therefore to examine both uterine and extra-uterine leiomyosarcoma for the presence of MDM2 gene copy number alterations by FISH.

Design: Archived formalin fixed paraffin embedded tissue blocks for 9 cases of uterine leiomyosarcoma and 8 cases of extra-uterine leiomyosarcoma were identified from case records. After deparaffinization and enzyme treatment, whole tissue cut sections of cases were incubated with a dual color FISH probe cocktail containing a labeled probe to the MDM2 genomic locus and a labeled probe to the centromeric region of chromosome 12 (SE12). High power images of probed sections were captured with a Metasystems image analysis system. MDM2 and SE12 FISH signals were then enumerated on 50 DAPI stained nuclei for each case and MDM2:SE12 ratios calculated. Tumors were scored for the presence of MDM2 amplification (MDM2:SE12 ratio ≥2.0) and MDM2 polysomy (MDM2 ≥ 3.0, MDM2:SE12 < 2.0).

Results: The average MDM2 signals, SE12 signals and MDM2:SE12 ratio (with ranges in parentheses) for the uterine leiomyosarcomas were 4.2 (2.4-10.1), 3.4 (2.0-6.0), and 1.2 (0.7-1.8), and for the extra-uterine leiomyosarcomas were 2.8 (1.8-4.2), 2.2 (1.7-2.9), and 1.3 (0.7-2.1), respectively. Low level MDM2 amplification was found in 2/8 (25%) extrauterine cases (MDM2:SE12 ratio 2.0 and 2.1) and 0/9 (0%) uterine cases. MDM2 polysomy was found in 1/8 (12.5%) extrauterine cases and 5/9 (55%) uterine cases.

Conclusions: Low level amplification of MDM2 and MDM2 polysomy were seen in extra-uterine and uterine leiomyosarcomas, respectively. The uterine leiomyosarcomas generally had higher MDM2 gene copies than the extra-uterine tumors, although a subset of the extra-uterine tumors exhibited low level amplification, which was not found in the uterine tumors. Caution should therefore be exercised in diagnostic MDM2 FISH evaluation of spindle cell tumors.

83 Diagnostic Utility of a Novel *ETV6/NTRK3* RT-PCR Assay and Break-Apart *ETV6* FISH Analysis in the Diagnosis of Congenital Fibrosarcoma and Cellular/Mixed Congenital Mesoblastic Nephroma

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Background: Congenital Fibrosarcoma (CFS) and Cellular Congenital Mesoblastic Nephroma (CMN) are characterized by *ETV6/NTRK3* gene fusions [t(12;15)(p13;q25)]. Both neoplasms occur chiefly in children; CFS (<2 yrs) and CMN (<6 months). As a diagnostic aid, and to determine prevalence of these translocations in CFS and cellular/mixed CMN, we developed novel RT-PCR and FISH-based approaches. This strategy is useful to: (1) test formalin-fixed, paraffin-embedded (FFPE) tissue specimens, (2) detect transcript variants in specimens with minute/poor quality RNA or small biopsies and (3) identify potential variant translocations or cryptic rearrangements of *ETV6*.

Design: A RT-PCR assay to detect *ETV6-NTRK3* transcripts (variants 1/2) was designed and tested on morphologically classic cases of CFS (12) and cellular/mixed CMN (12) from patients aged 4 days-17 months and 3 differentials (1 lipofibromatosis, 1 fibrous hamartoma of infancy, 1 desmoid tumor). Unstained FFPE sections from 10 of 12 CFS and all 12 CMN samples were tested by commercial break-apart FISH *ETV6* probe.

Results: An *ETV6-NTRK3* transcript was detected in 6/12 CFS (50%) and 2/12 CMN (17%) including 1 cellular CMN and 1 mixed CMN. Primers designed to detect a theoretical ETV6-NTRK3 variant 3 transcript amplified a novel sequence with homology to ETV6 and truncated NTRK3 (1 case). 3' RACE failed to amplify cases negative for the ETV6-NTRK3 variants 1/2 or reveal new *ETV6* fusion partners. The 3 differentials for CFS/CMN were negative for the fusion transcripts. FISH analysis (100 nuclei count) showed 100% correlation with RT-PCR results in cases with utilizable RNA and was also positive in 1 case with degraded RNA (non-diagnostic RT-PCR).

Conclusions: RT-PCR detection of ETV6-NTRK3 transcripts and *ETV6* FISH are reliable diagnostic adjuncts for CFS and cellular or mixed CMN. Confirmatory *ETV6* FISH analysis is valuable in cases with degraded or non-diagnostic RNA. This data suggests that the ETV6/NTRK3 transcript is more prevalent in CFS than cellular/mixed CMN. Since the *ETV6* rearrangement and ETV6-NTRK3 fusion was not detected in a subset of cases, cryptic/variant translocations, alternative fusion partners or completely novel molecular derangements are relevant considerations, and are subjects of on-going investigation in our laboratory.

84 Desmoid Tumors: Probing the Wnt Pathway

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Background: Aggressive fibromatosis (desmoid tumor) occurs sporadically or in the setting of Gardner syndrome, which is characterized by mutations in the adenomatous polyposis coli (*APC*) gene. The majority of desmoid tumors demonstrate nuclear accumulation of β-catenin, which is normally degraded by APC in the Wnt-signaling pathway. Recently there has been speculation that the extracolonic manifestations of Gardner syndrome may reflect a disorder of primary cilia, as ciliary function involves the Wnt pathway. End-binding protein 1 (EB1) is an APC-binding protein involved in intraflagellar transport, and normally binds APC in the region that is frequently lost in *APC* mutations. In vivo studies show that EB1 is normally present in fibroblasts and when absent, primary cilia assembly and ciliogenesis is attenuated. We compare immunohistochemical staining of β-catenin and EB1 in desmoid tumors, and examine immunostaining for cyclin D1 and WT1, which are also part of the Wnt pathway.

Design: A tissue microarray was constructed from 47 archival formalin-fixed, paraffin-embedded desmoid tumors resected from 35 patients, as well as other spindled cell tumors: malignant peripheral nerve sheath tumor (1), synovial sarcoma (4), gastrointestinal stromal tumor (5), solitary fibrous tumor (5), inflammatory myofibroblastic tumor (3), low-grade fibromyxoid sarcoma (2), and fibrosarcoma (2). Immunohistochemistry was performed for β-catenin, EB1, cyclin D1, and WT1. Results were recorded as nuclear versus cytoplasmic staining, or negative.

Results: There were 12 men and 23 women. Eight patients had Gardner syndrome, 21 tumors were intra-abdominal and 26 were extra-abdominal, and 11 patients had local recurrence. Nuclear β-catenin was present in 25 tumors (53%) and prominent cytoplasmic accumulation was seen in 16 tumors (34%), neither of which were seen in the 22 non-desmoids. Nuclear staining for EB1 was observed in 38 tumors (84%) and in two non-desmoid tumors (1 MPNST, 1 LGFMS). 24 (51%) desmoid tumors showed nuclear staining for cyclin D1. WT1 was negative in all desmoid tumors. There was no correlation between immunohistochemical staining and the evaluated clinical parameters, including Gardner syndrome.

Conclusions: The immunohistochemical expression of EB1 and cyclin D1 in desmoid tumors may reflect accumulation secondary to loss of APC and dysregulation of the Wnt pathway. EB1 accumulation may indicate an etiologic role for or at least shared signaling pathways with ciliary dysfunction. Nuclear β-catenin staining is known to be focal in practice, and EB1 immunohistochemistry may offer diagnostic utility.

85 The Analysis of Cytogenetics of Giant Cell Tumors

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Background: Multiple studies have identified the prevalence and clinical significance of a various genetic markers including telomeric association in giant cell tumors (GCTs). It is very important to analyze the DNA copy number change, to identify the molecular events in GCTs.

Design: In this study, we have used array-based comparative genomic hybridization (CGH) for high resolution mapping of copy number changes in 8 giant cell tumors.

Results: Primary bone tumors showed copy number gains in 12.2% of the genome and losses in 11.2%. Genetic instability, showing aberrations in more than 100 in 287 clones, were identified in 4 patients. Six clones showed high-level amplification in these 4 cases with genetic instability. They were *MYC* (8q24), *CREBBP* (16p13.3), *BRCA1* (17q21), *THRA* (17q11.2), *D19S238E* (19qtel), *TNFRSF6B* (DCR3; 20q13). Ten clones of *NRAS* (1p13.2), *D2S447* (2qtel), *D3S1274;ROBO1* (3p12-13), *D4S114* (4p16.3), *PIMI* (6p21.2), *MOS* (8q11), *HRAS* (11p15.5), *GLI* (12q13.2-13), *AKT1* (14q32), *D17S1670* (17q23) were commonly low in genetic instability cases. In telomeric area analyzed 73 clones, loss of *D2S447* (2qtel), and gain of *WI-6509* (11qtel) and *D19S238E* (19qtel) were observed. Our results of gain of MYC (8q24) gene was supported the findings of c-myc gene over-expression.

Conclusions: Gambri, et al has already reported c-myc mRNA was over-expressed in 38% of GCT. The loss of NRAS was observed in 6 cases (75%) out of 8 GCTs. NRAS mutations have detected prostate cancers before. However, there was no report the relashionship GCTs and NRAS before. Our present array-based CGH analysis indicated to 16 genes showing genetic instability, as the target gene on oncogenesis of GCTs.

86 Immunohistochemical and Cytogenetic Analyses of FGF23, and Chondrogenic and Osteogenic Transcription Factor Expression in Phosphaturic Mesenchymal Tumor

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Background: Phosphaturic mesenchymal tumor (PMT) is a rare mesenchymal neoplasm arising from both bone and soft tissue, which induces oncogenic osteomalacia (OO) as a paraneoplastic syndrome. Recently, FGF23 secreted by tumor cells is identified as a cause of OO; however, the mechanisms underlying the overexpression of FGF23 and the true nature of PMT tumor cells remain to be elucidated.

Design: Eight PMTs (6 from bone and 2 from soft tissue) with known OO, including 6 cases of mixed connective tissue variant (MCT) and 2 cases of osteoblastic variant (OB), were studied. Cytogenetic analysis was performed in 4 examples of MCT by dual-color chromogenic in situ hybridization (CISH) using chromosome 12 centromere (CEN12) and *FGF23* probes. FGF23 immunohistochemistry was undertaken, using two different antibodies. In order to better characterize PMT tumor cells, the expression status of essential transcription factors in chondrogenesis (Sox9) and osteogenesis (Runx2 and Osterix) were examined by immunohistochemistry (2+:positive cells>50%, 1+:positive cells<50%).

Results: Immunohistochemically, focal cytoplasmic staining of FGF23 was observed in all the PMTs, whichever antibody was used. No copy number gain or loss of *FGF23* was detected by CISH; tetrasomy 12 was however focally observed in 3 of 4 cases. Five of 6 MCTs showed higher level of immunoexpression of RUNX2 (2+) than Osterix (1+); on the contrary, both OBs labeled more for Osterix (2+) than Runx2 (1+). Sox9 expression was significantly lower than RUNX2 in MCTs (1+: 5/6 cases, 0: 1/5 cases). No Sox9 expression was observed in either OB.

Conclusions: FGF23 is a useful immunohistochemical marker for the diagnosis of PMT. The CISH analysis suggests the overexpression of FGF23 occur by mechanisms other than direct copy number alteration of *FGF23* gene, and that chromosomal instability involving chromosome 12 may play a role in MCT. The predominant expression of osteogenic markers may indicate that PMT possess an osteogenic potential that is only incompletely unfolded. Since Runx2 is known to be expressed prior to Osterix in bone development, the higher Runx2 expression level in MCT may reflect its remaining at earlier phase in osseous differentiation than OB.

87 FLI-1 Distinguishes Ewing Sarcoma from Small Cell Osteosarcoma and Mesenchymal Chondrosarcoma

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Background: Small cell osteosarcoma and mesenchymal chondrosarcoma are two primary bone tumors with a small round blue cell component which can mimic the appearance of Ewing sarcoma. Accurate diagnosis of these tumors on biopsy material is crucial, as optimal therapy differs according to tumor type. However, separating these entities by morphology alone can be challenging and all are positive for CD99. FLI-1 has been shown to be a useful marker for Ewing sarcoma, particularly when hematolymphoid markers are negative. Its staining patterns in small cell osteosarcoma and mesenchymal chondrosarcoma have however not been adequately characterized.

Design: Using a monoclonal FLI-1 antibody (BD Pharmingen G146-222), immunoreactivity in tumor cell nuclei was evaluated in 10 small cell osteosarcomas, 4 mesenchymal chondrosarcomas and 7 Ewing sarcomas with EWSR1-FLI1 fusion. Stromal endothelial cell served as an internal positive control. All cases were reviewed by institutional subspecialty bone and soft tissue pathologists and all cases diagnosed as small cell osteosarcoma on biopsy samples displayed conventional osteoblastic differentiation in the corresponding surgical resection specimens.

Results: None of the 10 small cell osteosarcomas nor the 4 mesenchymal chondrosarcomas exhibited detectable FLI-1 staining in the tumor cells, while all cases showed positive nuclear FLI-1 staining in the endothelial cells of the tumor stroma. In comparison, all 7 Ewing sarcomas showed moderate-strong nuclear FLI-1 staining in the tumor cells in addition to strong endothelial cell staining. Furthermore, FLI-1 positivity was not observed in a small series of other non-hematolymphoid small round blue cell tumors (4 rhabdomyosarcomas, 2 small cell carcinomas, 1 neuroblastoma and 1 desmoplastic small round cell tumor). In a series of 56 lymphoid neoplasms containing the more common B-cell and T-cell lymphoma types, only T-cell lymphoblastic lymphoma showed moderate-strong FLI-1 positivity.

Conclusions: In contrast to Ewing sarcoma with EWSR1-FLI1 fusion, tumor cells in small cell osteosarcoma and mesenchymal chondrosarcoma lack FLI-1 immunoreactivity. This test is therefore useful in the differential diagnosis of non-hematolymphoid small round blue cell tumors of the bone.

88 Differing Prognostic Significance of Tumor Associated Macrophages in Gastrointestinal Leiomyosarcomas and Gastrointestinal Stromal Tumors

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Background: Gastrointestinal stromal tumors (GIST) and leiomyosarcomas (LMS) are two mesenchymal tumors that can arise anywhere along the gastrointestinal tract. While they were initially thought to represent the same disease, it is now clear that they are biologically distinct entities. We have previously demonstrated the prognostic significance of tumor associated macrophages in a series of predominantly soft tissue based LMS. The extent and the importance of stromal macrophage infiltrate in GIST and gastrointestinal LMS however remains uncharacterized.

Design: Using a series of tissue microarrays that contain 419 GIST and 63 gastrointestinal LMS with long term follow-up, we determined the density of the macrophages in each 0.6 mm tumor cores with the macrophage marker CD163 and examined its prognostic significance by Kaplan-Meier analysis.

Results: CD163-positive stromal macrophages were present in nearly all GIST and all gastrointestinal LMS examined. While macrophage density varied between tumors, LMS overall showed greater amount of macrophages in their stroma (median density of 45 macrophages per core) when compared to GIST (median density of 21 macrophages per core). Kaplan-Meier survival analysis showed no significant differences between subgroups of GIST with different macrophage densities. In contrast, dense stromal macrophage infiltrates (> 70 macrophages per core) in gastrointestinal LMS was associated with decreased 5-year overall survival (20% versus 55%, p=0.009) with the difference being significant up to 20-year follow-up (p=0.011). Presence of tumor necrosis was found to be a significant negative prognosticator for GIST (p=0.008) but not for gastrointestinal LMS (p=0.512).

Conclusions: We have observed a significant association between dense stromal macrophage infiltrates and decreased overall survival in gastrointestinal LMS but not in GIST. This finding likely reflects known differences in the biology between them. It also provides further support to the importance of tumor associated macrophages in the progression of LMS, irrespective of the site of origin.

89 Morphologic and Immunohistochemical Characterization of a Series of Clinically Atypical Skull Base Chordomas in the Paediatric Population

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Background: Chordomas are neoplasms that are hypothesized to arise from prexisting notochordal proliferations. While it is primarily a disease of adults, a subset of chordomas appears during childhood. These paediatric chordomas tend to occur in the skull base and a minority display unusually aggressive clinical behaviour with a propensity for metastases.

Design: We retrospectively examined a series of 22 paediatric chordomas that exhibited unusual pattern of disease progression including the presence of aggressive local growth and/or development of distant metastases. The aim is of the study is to identify specific histologic and immunohistochemical features that are common to this group of clinically atypical chordomas.

Results: The median age of the patients was 5.5 year (1 to 17 year). All of the primary tumors were located in the skull base region. Upon review, 17 of the 22 tumors demonstrated a morphologic appearance and/or immunohistochemical profile (positive for keratin and brachyury) consistent with chordoma, while the 5 remaining cases were poorly differentiated epithelioid neoplasms that strongly expressed keratin but lacked staining for brachyury. Of the 17 review-confirmed chordomas, only 3 chordomas displayed conventional morphology, while the majority (12 of 17 chordomas) showed increased cellularity in a large portion of the tumor (cellular variant). One remaining case showed focal dedifferentiation. A collagenous/demoplastic-type stromal background was seen in nearly half of these cases. Six of the 8 chordomas with positive Ki-67 stains showed a high proliferation index (> 10%) across multiple high power fields.

Conclusions: We have identified several important histologic features including increased cellularity, demoplastic-type stroma, and high proliferation index that are commonly encountered in clinically aggressive paediatric chordomas. Additionally, we found a small subset of poorly differentiated keratin-expressing and brachyury negative epithelioid neoplasms of the skull base that warrant more detailed characterization.

90 Prognostic, Biological, and Potential Therapeutic Implications of Gene Amplification-Driven Skp2 Overexpression in Myxofibrosarcoma

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Background: The tumorigenesis and prognostication of myxofibrosarcoma (MFS) remains obscured, for which we previously reported Skp2 overexpression as an adverse prognosticator (*Clin Cancer Res 2006*). Harboring *Skp2* gene on 5p13, chromosome (Chr) arm 5p is frequently gained in copy number among various sarcomas, while its specific oncogenes have never been analyzed for MFS.

Design: To search oncogenes on Chr5**,** we used 385K array comparative genomic hybridization (aCGH) to profile DNA copy number alterations of 12 samples and 2 MFS cell lines (NMFH1, OH931), with special attention to *Skp2*. Another 82 primary tumors were measured for *Skp2* gene dosage by real-time PCR and immunostained for Skp2. MFS cells were transiently transfected with Skp2-specific cDNA and siRNA or treated with bortezomib (a proteasome inhibitor) to analyze the effects on various cellular processes, Skp2 abundance, and/or cytotoxicity.

Results: aCGH identified nonrandom 5p gains in NMFH1 and 5 grade II or III tumors with 2 recurrent amplicons mapped to 5p15.1-15.2 and 5p12-13.3. The latter showed DNA gain in the *Skp2* locus. Present in 31 cases (38%), *Skp2* amplification was strongly associated with deep location, advanced stages, higher grades, large size, tumor necrosis, and mitotic activity (all $p<0.01$). While gene status correlated with Skp2 abundance ($p<0.001$), 5 of 35 MFSs with Skp2 overexpression did not show gene amplification, corroborating lack of this aberration in Skp2-overexpressing OH931 cells. *Skp2* amplification was independently predictive of adverse outcomes for local recurrence-free survival (p=0.0179, RR=2.159) along with margin status, for metastasis-free survival (p=0.0011, RR=5.064) with higher grades, and for disease-specific survival (p=0.0004, RR=10.919) with proximal location. Silencing of Skp2 expression in both MFS cell lines attenuated cell proliferation and retarded cell migration, which were reversed by ectopic Skp2 overexpression. Treatment with bortezomib at a low concentration (10nM) could remarkably suppress Skp2 expression, induce activation of caspases, and, as compared to fibroblasts, result in apparently declined cell viability in both NMFH1 and OH931 cells.

Conclusions: *Skp2* amplification*,* the predominant but not the sole cause of protein overexpression in MFS, plays a critical role in tumor aggressiveness. The Skp2-suppressing effect of bortezomib and the relative cytotoxic sensitivity of MFS indicate the potentiality of Skp2 and ubiquitin-proteasome pathway as therapeutic targets.

91 MicroGIST Genomic Aberrations Highlight Early Mechanisms in GIST Pathogenesis

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Background: MicroGISTs (0.1-1 cm in diameter) are on a biological continuum with larger clinically-significant GISTs, and hence provide compelling models in which to study early oncogenic aberrations. However, in contrast to clinical GISTs (annual incidence ~15 cases per million), most microGISTs (identified in 20-30% of the population) involute, becoming hyalinized and calcified, and rarely progress to symptomatic GIST. To determine biological relationships, between micro and macro GISTs, we characterized genomic alterations by sequencing and array CGH analyses.

Design: Analyses were performed on 32 microGISTs, ranging from 0.1 to 1 cm in diameter, with variable hyalinization, of which 28 were spindle-cell, and 2 each were epithelioid or mixed type. DNAs from microdissected microGISTs and adjacent normal tissues were sequenced for *KIT* and *PDGFRA* mutations and were analyzed in genome-wide screens by Agilent 400K array CGH, using four whole genome amplification pools from each sample.

Results: *KIT* mutations were identified in 26 microGISTs (81%) and *PDGFRA* exon 12 mutation was found in one microGIST. The *KIT* mutations were similar in type and exonic distribution to those reported in clinically-significant metastatic GISTs [e.g. exon 9 mutation in 4 cases; exon 13 mutation in 2 cases; exon 11 deletions involving amino acid 558 in 7 cases]. Novel mutations, never before reported in GIST, involving exons 9, 11, 13 and 18, were demonstrated in four microGISTs. Array CGH demonstrated monosomy 14 in three microGISTs, and the monosomy 14 in these cases (evaluated by interphase FISH) involved both hyalinized and cellular regions. Other array CGH aberrations, found in one microGIST, each, were monosomies 10, 15 and 22, and trisomy 2.

Conclusions: Certain *KIT* mutations are known to correlate with unfavorable natural history in GIST [exon 9, exon 11 amino acid 557-558 deletions]. Our studies show that these same mutations are frequent in microGISTs that rarely progress to clinically problematic lesions. Therefore, such mutations are not, in and of themselves, biological mechanisms of aggressive behavior. Likewise, we show that known recurrent cytogenetic aberrations in GIST, particularly monosomy 14, are well-entrenched in microGISTs with very low-likelihood of clinical progression, and are therefore necessary, but not sufficient, events in genetic progression towards malignancy.

92 Ezrin Immunoexpression in Bone Sarcomas (Osteo-Chondrosarcomas and Ewing/PNET)

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Background: Ezrin is a cytoskeleton protein involved in metastatic development. Ezrin expression has been described in osteosarcomas and studies have recommended the utility of this protein as a tool to differentiate between chondrosarcomas and chondroblastic osteosarcomas (Salas S, de Pinieux G, Gomez-Brouchet A et al. Virchows Arch. 2009; 454(1):81-7). Ezrin expression in others bone tumors (Ewing/PNET) has been reported in isolated cases.

Design: A total of 397 bone tumors (33 osteosarcomas, 23 chondrosarcomas and 341 Ewing/PNET) were immunostained with ezrin antibody (clone 3C12, dilution 1:250). The immunoexpression was scored as negative; poor (+) moderate (++) strong (+++) cytoplasmic/membranous positivity.

Results: Chondrosarcoma showed low expression of ezrin (30%) and within the positive cases revealed predominantly high grade (Grade III) at histopathological level. Ezrin immunoexpression in osteosarcoma was high (69%), however chondroblastic osteosarcoma did not reveal ezrin expression; therefore, ezrin antibody probably cannot distinguish chondroblastic osteosarcoma from chondrosarcoma as proposed in previous publications. Ewing/PNET revealed ezrin expression in 40.7% of the tumors, with higher expression shown in atypical Ewing sarcoma (58.9%) and PNET (58.6%) compared with conventional Ewing (30%).

Conclusions: Ezrin immunoexpression is heterogeneous in bone tumors and immunostaining did not offer any diagnostic clue in the differential diagnosis of chondroblastic tumor. Additionally, Ewing/PNET frequently reveals ezrin expression. The protein expression seems to be associated with the histological grade, being higher in morphologically high-grade tumors. As previously reported, ezrin could be a molecular

therapeutic target in bone tumors, but further studies are required to assess prognostic value. **Support:** EuroBonet (European network of excellence**)**. Contract No. 018814

93 Dedifferentiated Liposarcoma with "Homologous" Lipoblastic (Pleomorphic Liposarcoma-Like) Differentiation: Clinicopathologic and Molecular Analysis

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Background: Dedifferentiated liposarcoma (DDLPS) is a malignant adipocytic neoplasm defined as transition from well-differentiated liposarcoma (WDLPS) to a non-lipogenic sarcoma. Heterologous differentiation is seen in 5% of DDLPS, usually with myogenic or osteo/chondrosarcomatous elements; adipocytic differentiation in the high grade component is incompatible with the current definition of DDLPS. Pleomorphic liposarcoma (pLPS) is a high grade sarcoma containing lipoblasts. pLPS can be indistinguishable from DDLPS, except for the presence of lipoblasts in pLPS and WDLPS areas in DDLPS.

Design: We evaluated 10 unusual liposarcomas: 9 WDLPS/DDLPS with high grade areas showing lipoblastic differentiation and 1 resembling inflammatory "MFH" with scattered lipoblasts. Clinical and histologic features were reviewed. Immunohistochemistry for MDM2 and CDK4 was performed. Amplification of 12q15 was studied by FISH analysis of the HMGA2 locus.

Results: Tumors arose in the retroperitoneum (7), proximal lower extremity (2) and neck (1) of 7 males and 3 females (median 67 yrs, range 49-76). Size ranged from 9-32 cm. In 3 cases there was abrupt transition between WDLPS/DDLPS and sheets of pleomorphic lipoblasts, indistinguishable from pLPS. Four cases consisted of otherwise typical DDLPS (with adjacent WDLPS), except for the focal presence of lipoblasts in the high grade component. One case contained both non-lipogenic spindle cell areas and an inflammatory "MFH"-like component with numerous admixed lipoblasts. Two cases were composed exclusively of pLPS-like areas developing in one of the recurrences of DDLPS. Two also showed heterologous smooth muscle differentiation. MDM2 and CDK4 were positive in both the DDLPS and pLPS-like components in 8/8 and 7/8 cases, respectively. Karyotyping, available for 1 case, showed giant marker chromosomes. FISH analysis showed high-level amplification of 12q15 in 3 cases tested so far. Follow-up, available on 8 patients, ranged from 21-196 mos (median 42). Four patients developed local recurrences (multiple in 3), and 2 lung metastases. Thus far, 5 patients died of disease, 1 died of unknown cause and 2 are alive with no evidence of disease.

Conclusions: DDLPS can show lipoblastic differentiation in the high grade component, resulting in areas indistinguishable from pLPS. Available clinical and molecular data support the notion of "homologous" lipoblastic differentiation in DDLPS, rather than mixed-type liposarcomas.

94 p63 Immunohistochemistry Is a Useful Marker for Muscle Differentiation

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Background: p63, a member of the p53 gene family, is a selective nuclear marker of myoepithelial cells. Antibody against p63 is frequently used in the diagnosis of prostate carcinoma, as well as in the identification of myoepithelial cells in other tissues including the breast. p63 has also been shown to be expressed in squamous cell carcinoma, metaplastic breast carcinoma, sarcomatoid carcinoma, and giant cell tumor of bone. Recently, it was found that all p53 family members are involved in regulating the process of muscle differentiation through the retinoblastoma protein. Ablation of these p53 family functions blocks the differentiation program and promotes malignant transformation by enabling cooperating oncogenes to transform myoblasts.

Design: Immunohistochemical staining for p63 was performed on paraffin sections of 35 rhabdomyosarcomas, 5 leiomyomas, 5 leiomyosarcomas, 5 rhabdomyomas, 5 rhabdomyomatous Wilms' tumors, 3 cases of normal cardiac muscle and 1 medullomyoblastoma. Each case was also stained with desmin and myogenin for comparison purposes. Unlike the nuclear staining scored in myoepithelial cells, only cytoplasmic staining for p63 was considered positive.

Results: Thirty-four of 35 cases of rhabdomyosarcoma showed cytoplasmic p63 staining. Thirteen of these cases displayed exquisite definition of the cross-striations. In addition, 5/5 cases of rhabdomyoma, 5/5 cases of rhabdomyomatous Wilms' tumor, and 1/1 case of medullomyoblastoma exhibited cytoplasmic p63 staining. Cardiac muscle samples (3/3) also demonstrated positive cytoplasmic staining and distinct cross striations. Smooth muscle tumors exhibited only very focal and faint cytoplasmic staining in 5/5 leiomyomas and 4/5 leiomyosarcomas. p63 cytoplasmic staining was more intense and highlighted the cross striations with superior definition than desmin.

Conclusions: The p63 immunostain is a sensitive marker for skeletal muscle differentiation and highlights the cross-striations of strap cells with exceptional definition, far superior to desmin. p63 appears to localize to the Z bands of skeletal and cardiac muscle, while smooth muscle, which lacks cross-striations, does not stain as intensely. Ultrastructural immunohistochemical examination of p63 stained sections to aid in protein localization is underway.

95 Characterization of Rare Chondrosarcoma Subtypes

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Background: Besides conventional chondrosarcoma several rare chondrosarcoma subtypes are defined, together constituting 10-15% of all chondrosarcomas. Clear cell chondrosarcoma (CCS) (2%) is a low-grade malignant tumor which rarely metastasizes, but commonly recurs after curettage. About 15% of the patients die as a result of the disease. Mesenchymal chondrosarcoma (MCS) (2%) is a highly malignant lesion occurring in the bone and soft tissue of relatively young patients. The tumor consists of differentiated cartilage mixed with undifferentiated small round cells and usually follows an aggressive course with a high rate of distant metastases and a 5-year overall survival of 55%. Dedifferentiated chondrosarcoma (DDCS) (10%) is a tumor containing a high-grade non-cartilaginous (DD; dedifferentiated) sarcoma and a usually low-grade malignant (WD; well-differentiated) cartilage-forming tumor, with a sharply defined junction between the two components. It bears a poor prognosis and no targets for therapy have been reported so far. The lack of efficacious treatment of these rare tumors emphasizes the need to learn more about their characteristics and to unravel potential targets for therapy.

Design: We constructed tissue microarrays (TMAs) containing 2mm cores of 45 DDCS (WD and DD), 24 CCS, and 25 MCS, in triplicate. The TMAs are immunohistochemically stained for estrogen-signaling molecules, growth plate-signaling molecules, and other molecules which might be potential targets for therapy. In addition, from the paraffin-embedded tissue genomic information was gathered using Agilent 44K oligo arrays.

Results: For Cox-2, 30% of the WD components of DDCS were positive. Almost all DD, CCS, and MCS were negative. In MCS 88% of the small cells and 32% of the cartilage were positive for Bcl2. In CCS, WD, and DD 48%, 4%, and 12% were positive, respectively. We demonstrated the presence of ESR1 and aromatase protein in the majority of tumors in all subtypes. ArrayCGH showed that the two components of DDCS share similar aberrations, with additional aberrations in the DD, e.g. a homozygous deletion of p16.

Conclusions: As most of the tumors are negative for Cox-2, celecoxib treatment is not recommended. However, application of Bcl2 inhibitors might chemosensitize MCS, and the presence of ESR1 and aromatase support a possible effect of anti-estrogen treatment in all subtypes.

96 Malignant Peripheral Nerve Sheath Tumors of the Uterine Cervix: Support for a Low-Grade Variant with Possible Derivation from CD34-Positive Endoneurial Cells

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Background: Malignant peripheral nerve sheath tumors (MPNST) of the uterine cervix are rare and incompletely characterized. As at other sites, the derivation of cervicovaginal MPNSTs is poorly understood and the relative contribution of endoneurial elements and conventional Schwannian cells is unknown.

Design: Clinical features, morphology, immunohistochemical staining profile, ultrastructural features, and follow up were obtained on three primary cervical MPNST.

Results: Two of three patients with cervical MPNST were <35 years of age. One patient had a history of multiple schwannomas and dermatofibrosarcoma protuberans (case 2), but none had a diagnosis of neurofibromatosis. Tumor histology, immunohistochemical staining for S100 protein and CD34, ultrastructural morphology, and follow up is presented in Table 1. All tumors were negative for desmin, h-caldesmon, myogenin, CKmix, ER, PR, CD10, HMB45, and Melan A, but showed strong, diffuse staining for vimentin.

	Morphology	CD34	S100 protein	EM	Behavior
Case 1 (32 yo)	Cytologically bland spindled cells with intervening bands of collagen; low mitotic index	Strong, diffuse (~70%)	Strong, moderate (~40%)	Technically poor study	Polypectomy with positive margins; complete excision on subsequent trachelectomy with no evidence recurrence at 34 months
Case 2 (60 yo)	Spindled cells with mild nuclear pleomorphism; mitotic figures readily identified	Strong, moderate (~40%)	Absent	Mixed cell population including fibroblast-like cells similar to endoneurium	Involvement of vaginal tissues and pelvic side wall at initial hysterectomy; local recurrence at 9 months
Case 3 (25 yo)	Atypical proliferation of spindled cells in tight fascicles; frequent mitotic figures	Strong, focal (<10%)	Strong, diffuse (~60%)	Technically poor study	Extensive pelvic recurrence with involvement of ovaries, small bowel, and omentum at 9 months

Conclusions: Focal to diffuse strong CD34 staining in all 3 cervicovaginal MPNST in conjunction with ultrastructural features of endoneurial cells in these tumors suggests possible mixed derivation from both conventional Schwannian elements and CD34-positive endoneurial cells. Further studies are warranted to determine whether extent of CD34-positive endoneurial differentiation impacts prognosis in MPNST.

97 Expression of Group I Leiomyosarcoma Markers by Gene Expression Analysis and Immunohistochemistry in a Subset of Undifferentiated Pleomorphic Sarcomas

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Background: Leiomyosarcomas (LMS) form a broad group of tumors that are defined by relatively subjective morphologic criteria and/or reactivity for actin or desmin. The distinction from undifferentiated pleomorphic sarcoma (UPS) can be problematic. We recently described 3 novel distinct LMS subtypes based on gene expression profiling and array comparative genomic hybridization that are distributed equally over LMS of GYN and non-GYN origin. The Group I subtype shows an improved disease-specific survival compared to the other 2 groups that is independent of histologic grade (Beck et al., *Oncogene* 2009 *in press*). Group I comprises ~25% of all LMS and is defined by a shared pattern of gene expression, a distinct pattern of genomic changes, and reactivity

for 5 immunohistochemistry (IHC) markers (ACTG2, CASQ2, CFL2, MYLK, SLMAP) that have not been well characterized in sarcoma. Here we provide a characterization of these 5 markers across a wide range of sarcomas with a focus on UPS. In addition we examine the gene expression profiles of 29 UPS analyzed on microarrays for LMS subset specific gene sets.

Design: IHC staining with ACTG2, CASQ2, CLF2, MYLK, and SLMAP was performed on tissue arrays containing 565 sarcomas and benign soft tissue processes from 44 diagnostic entities. Staining was scored as strongly positive, weak/absent, or uninterpretable. Only cores for which ≥3/5 stains were interpretable were included in analysis. The expression of the top 500 genes from the Group 1 LMS expression signature was evaluated in 29 cases of UPS by 44,000 spot cDNA microarrays.

Results: IHC staining revealed strong immunopositivity for ≥3/5 Group I markers in 32% of LMS (n=59), 5% of UPS (n=57), 22% of leiomyoma (n=22), 16% of gastrointestinal stromal tumor (n=43), and 18% of endometrial stromal sarcoma (n=11). Other soft tissue tumor types had negligible staining. Unsupervised hierarchical clustering of the 29 UPS expression profiled by cDNA microarrays revealed that 2 (7%) had coordinated high levels of expression of genes from the Group I LMS signature.

Conclusions: The Group I LMS IHC markers ACTG2, CASQ2, CLF2, MYLK, and SLMAP show specificity for a subset of LMS when compared to other sarcomas and benign entities and could be used as differential diagnostic tools. A subset of UPS demonstrates Group I LMS markers by microarray analysis and IHC. These markers may define a population of Group 1 LMS-derived UPS with improved prognosis and could suggest future therapeutic targets.

98 Functional and Phenotypical Analysis of 19 EuroBoNet Osteosarcoma Cell Lines

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Background: Recently the EuroBoNet consortium reported the genetic characterization of 36 bone tumour cell lines (DOI: 10.1002/gcc.20717). Here we present the functional analysis of all the osteosarcoma cell lines included there (n=19) regarding their histology, differentiation capacity and tumuorigenicity to identify cell lines that could be considered as representative images of the parental tumours which would be useful as *in vitro* and *in vivo* models to study osteosarcoma.

Design: For functional characterization a subset of the cell cultures was pushed towards differentiation into osteoblasts, adipocytes, and chondrocytes using differentiation specific culturing conditions. Furthermore all cell lines were injected subcutaneously and intra muscularly into nude mice to assay their *in vivo* tumour formation capacity as well as for phenotypical analysis of the tumours. All formed xenografts were further characterized histologically and immunohistochemically using markers involved in differential differentiation (osteonectin, osteocalcin, ALP, cytokeratin, EMA, SMA, desmin and LCA), growth (CD99, BCL2, caveolin1, Ki-67, p53, p16, p21, EGFR, CD34, Her2 and TPD52) and invasion/migration (ezrin, vimentin, e-cadherin and CD31) of osteosarcoma.

Results: 12/16 (75%) cell lines showed adipogenic differentiation of which 8 (8/16, 50%) could also differentiate towards osteoblasts. Only 1/4 (25%) cell lines assayed showed chondrogenic differentiation, interestingly this cell line was not able to differentiate towards adipocytes or osteoblasts. About half of the cell lines (11/19, 58%) produced tumours *in vivo* after subcutaneous and/or intra muscular injections. All xenografts showed a protein expression pattern representative of high grade osteosarcoma. Both adjacent invasion and lung metastases were observed in some cell lines.

Conclusions: The use of cell lines, especially in cancer research, is of high importance. Questioning their representativeness of clinical tumours should not be addressed by focusing on their genetics exclusively. This study has established a spectrum of osteosarcoma cell lines that robustly represent clinical osteosarcoma. Together with the previously characterized genetic attributes we now have a tool to study the association between genotype and phenotype in a well defined model.

99 Distribution of Sarcoma Histotypes and Subtypes in Selected Regions of France and Italy: A Report from CONTICANET Network of Excellence

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Background: Soft tissue tumours represents a broad and morphologically heterogeneous group of neoplasms accounting for less than 1 % of malignancies. The distribution of histological types and subtypes has significantly changed over the time as a result of the evolution of classification schemes as well as of the lack of diagnostic expertise in non referral centers.

Design: All soft tissue and visceral sarcomas occurring in patients living in 3 european regions (Veneto Italy, Aquitaine and Rhone-Alpes, France) have been collected prospectively during 2007. 804 cases were reviewed by specialist soft tissue pathologists and reclassified according to the 2002 WHO classification in order to analyzed the distribution of the histological type and subtypes.

Results: There were 526 soft tissue sarcomas and 278 visceral sarcomas. Among the soft tissue sarcomas, the most frequent histotypes were liposarcoma (23,6%), leiomyosarcoma (13,9%) sarcoma NOS (13,3%). Well differentiated liposarcoma accounted for about half of liposarcoma (45,7%), followed by dedifferentiated liposarcoma (25,6%), myxoid/round cells liposarcoma (13,9%) and pleomorphic liposarcoma (5.4%). Rarer histotypes included angiosarcoma (4,6%), rhabdomyosarcoma (3.8%), synovial sarcoma (2.7%), Ewing's sarcoma/PNET (2.3%), MPNST (1.3%). respectively. Undifferentiated pleomorphic sarcomas (ex-MFH) accounted only for 3.8% of all cases. Among the visceral tumors GISTs was the most frequent histology (62,9%) followed by leiomyosarcoma (15,5%).

Conclusions: Approximately 13% of sarcomas remains unclassifiable even when reviewed by an expert panel of soft tissue pathologists. At variance with recently published data undifferentiated pleomorphic sarcomas accounts for less than 4%, possibly reflecting more accurate classification. Dedifferentiated liposarcoma appears to be the second most frequent liposarcoma subtype reflecting the decline of MFH. GIST is the most frequent sarcoma type at visceral sites.

100 Expression of Heat Shock Proteins in Osteosarcomas

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Background: Heat shock proteins (HSPs) protect cells against stress-associated injury and are overexpressed in several malignant tumors. We aimed to investigate the potential role of HSP27, HSP60 and HSP70 in conventional and low grade central osteosarcoma (OSA).

Design: Expression of HSP27, HSP60 and HSP70 was analyzed using immunohistochemistry on tissue section from 52 cases of conventional OSA and 21 cases of low grade central OSA. Conventional OSA consisted of osteoblastic (n=45) and non-osteoblastic [chondroblastic (n=4), fibroblastic (n=2) and telangiectatic (n=1)]. We evaluated the expression of each protein and examined its relationship with clinicopathologic parameters.

Results: We classified the cases in which more than 10% of tumor cells were positive into the high expression group. The high expression rate in osteoblastic conventional OSA was 28.9% (HSP27), 93.3% (HSP60) and 88.9% (HSP70). The rate in non-osteoblastic conventional OSA was 71.4% (HSP27), 85.7% (HSP60) and 85.7% (HSP70). The high expression rate in low grade central OSA was 4.8% (HSP27), 85.7% (HSP60) and 14.3% (HSP70). The expression of HSP27 and HSP70 in conventional OSA was significantly higher than that in low grade central OSA (P=0.008 and 0.001, respectively). In conventional OSA, higher expression of HSP27 was significantly related to distant metastasis (P=0.034) and histological subtype [osteoblastic vs. non-osteoblastic (P=0.041)]. The expression of HSP60 and HSP70 was not significantly related to any clinicopathologic parameters.

Conclusions: HSP70 and HSP27 may be used as a differential marker to distinguish conventional OSA and low grade central OSA. HSP 27 may be used as a possible prognostic marker in conventional osteosarcoma cases.

101 Cytogenetic and Immunohistochemical Analyses of c-Met in Peripheral Nerve Sheath Tumors

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Background: Malignant peripheral nerve sheath tumor (MPNST) is an aggressive sarcoma with only limited treatment options. It often develops from neurofibroma (NF) either sporadically or in the setting of neurofibromatosis type 1 (NF1). c-Met proto-oncogene (c-Met) is a tyrosine kinase receptor, whose amplification and overexpression is known to be associated with tumor progression of several types of neoplasms. Recent array-based comparative genomic hybridization analysis revealed *c-Met* amplification in MPNSTs. In order to clarify the role of c-Met in the development of MPNST, benign peripheral nerve sheath tumors and MPNSTs were examined by brightfield double in situ hybridization (BDISH) for *c-Met* gene status, and by immunohistochemistry for the protein expression.

Design: Formalin-fixed paraffin-embedded tissue samples (FFPEs) from 8 cases of MPNST (3 NF1-associated, 5 sporadic), 5 cases each of NF1-associated- and unassociated-NF, and 3 cases of schwannoma (SCH) were studied. The BDISH assay was performed with *c-Met* and chromosome 7 centromere (CEN7) probes, visualized respectively by fast red and silver acetate. The *c-Met* and CEN7 signals in 60 nuclei were counted per sample. Immunohistochemistry was performed using anti-c-Met monoclonal antibody; c-Met overexpression was defined as definite cytoplasmic staining over 50% of tumor cells.

Results: MPNSTs frequently showed amplified signals of both *c-Met* and CEN7 with a range of 1.9-4.3 signals/nuclei (mean 2.6) for *c-Met* and 1.8-4.2 (mean 2.6) for CEN7, which indicated the polysomy of chromosome 7. No selective *c-Met* amplification was identified in MPNSTs (*c-Met*/CEN7; 0.9-1.1, mean 1.0). The polysomy 7 was also seen in SCHs (3/3 cases, *c-Met*; mean 2.3, CEN7; mean 2.1); but it was seldom observed in NFs (*c-Met*; 1.6-1.8, mean 1.7, CEN7; 1.5-1.9, mean 1.7) regardless of NF1 status. Immunohistochemically, most MPNSTs overexpressed c-Met protein (7/8 cases), whereas the overexpression was rarely seen in NFs and SCHs (1/13 cases).

Conclusions: Our result suggests that polysomy 7 be acquired during malignant transformation of NF. The detection of this cytogenetic abnormality by BDISH may thus be a useful adjunct in differentiating between NF and MPNST in morphologically challenging cases. Although no c-Met amplification was found in MPNST, polysomy 7 may be one of the genetic mechanisms by which c-Met overexpression is induced in this sarcoma.

102 Osteosarcoma of the Jaw Bones: A Clinicopathologic Study of 44 Cases

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Background: Osteosarcoma of the jaw bones has been considered to show clinicopathologically distinctive features, namely, generally affecting older patients,

frequently showing low grade histology with predominance of chondroblastic type, and generally better prognosis.
Design: Forty four cases of osteosarcoma of the jaw bones were retrieved from the surgical pathology file. Clinical and roentgenologic findings, operational charts, description of the gross materials and follow up information were reviewed for each case. H & E stained histologic sections were available for all tumors.
Results: Of the 44 patients with osteosarcoma of the jaw bones, there were 3 cases (maxilla of a 18-year-old female, mandible in a 32-year-old female, maxilla in a 59-year-old female) of postradiation osteosarcoma, and 3 cases (maxilla in a 49-year-old female, maxilla in a 61-year-old male, mandible in a 37-year-old female with Albright syndrome) of osteosarcoma secondary to fibrous dysplasia. Residual 38 cases of the primary osteosarcoma of the jaw bones showed the age distribution from 10 to 82 (average 44.89) years, included 20 females and 18 males with 29 mandibular (18 in the body, 2 in the angle, and 9 in the ascending ramus) and 9 maxillary (3 in the antrum, 5 in the alveolar ridge, and 1 in the palate) lesions. Roentgenologically, the lesion was generally an ill-defined area suggesting malignancy, with lytic and/or sclerotic changes. Mandibular lesions frequently showed periosteal reaction including spicules. Predominant histologic features were osteoblastic in 13 tumors, chondroblastic in 9 tumors, and fibroblastic in 16 tumors. These 3 histologic types, however, frequently coexisted even in the same histologic specimen of a tumor. Fifteen tumors are low grade (grade 1 in 1 tumor, grade 2 in 14 tumors) and 23 were high grade (grade 3 in 19 tumors, grade 4 in 4 tumors). All patients underwent resection of the tumor, and local recurence of the tumor and metastasis to the lung were main complication.
Conclusions: (1) Osteosarcoma of the jaw bones can show a wide histologic spectrum seen in conventional osteosarcoma in the long bones, with high incidence of high grade (grade 3 + grade 4) tumors than low grade (grade 1 + grae 2) tumors. (2) Although incidence of chondroblastic type is higher than in other locations, high grade osteoblastic and fibroblastic pattern is more common in osteosarcoma affecting the jaw bones.

103 Differential Expression of Proteins Regulating Lipogenesis in Liposarcoma

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Background: PPAR-gamma, a transcription factor important in adipocyte differentiation, has been shown to be expressed in all subtypes of liposarcoma, and treatment with PPAR agonists induces differentiation of liposarcoma cells in vitro. These observations suggest that signaling pathways involved in adipocyte differentiation might also be important in liposarcomas. We hypothesized that signaling pathways that inhibit adipocyte differentiation would be more prominent in more aggressive tumor types.
Design: We constructed a liposarcoma tissue microarray that included well-differentiated, de-differentiated, myxoid, round-cell and pleomorphic liposarcomas. Using immunohistochemistry, we determined the expression of components of signaling pathways known to inhibit adipocyte development, including Wnt10b, ß-catenin, Notch1 and DLK1.
Results: Of 22 well-differentiated liposarcomas, none expressed Wnt10b or DLK1, and only 1 (5%) was positive for nuclear Notch 1. Well differentiated liposarcoma may progress to de-differentiated liposarcoma. Of 12 de-differentiated liposarcomas, 7 (58%) had nuclear Notch 1, and none expressed Wnt10b or DLK1. Of 16 myxoid liposarcomas, 1 (6%) expressed DLK1, 3 (19%) expressed Wnt10b, and 10 (62%) were positive for nuclear Notch 1. Myxoid liposarcoma may progress to a round cell phenotype, and 6 of the myxoid tumors positive for Notch 1 contained a transitional or round cell component. Of 8 round cell tumors, 1 (13%) expressed DLK, 3 (38%) expressed Wnt 10b and all 8 were positive for nuclear Notch 1. Pleomorphic liposarcomas are the most aggressive liposarcoma, and they had significantly higher rates of proliferation and greater nuclear staining of p53 than other tumor types. Of 12 pleomorphic liposarcomas, only 2 (17%) were positive for Wnt10b, but 10 (83%) were positive for DLK and nuclear Notch1. No tumors were positive for ß-catenin.
Conclusions: Overall, these results suggest that more aggressive, less differentiated types of tumors are more likely to express inhibitors of adipocyte differentiation, with Notch 1 activation frequently occurring as tumors progress to a more aggressive phenotype. In contrast, DLK was expressed only in pleomorphic tumors. Despite a role in inhibiting adipocyte differentiation during development, Wnt10b was not detected in the vast majority of liposarcomas.

104 Parachordoma Involving Soft Tissue and Bone: A Distinctive Subgroup of Myoepithelioma or Extra-Axial Chordoma?

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Background: Parachordoma is a rare bone and soft tissue tumor of uncertain lineage that occurs in non-axial locations. Although it was described several decades ago it remains enigmatic.
Design: We retrieved 16 cases from our archives using the search terms "parachordoma" and "soft tissue myoepithelioma". Criteria for inclusion in the study included close morphologic resemblance to axial chordoma in the absence of known chordoma or carcinoma; 8 cases fulfilled these criteria. Immunohistochemical stains were performed. Corresponding clinical information was tabulated.
Results: Clinical data, available in 6/8 cases, showed there were 4 males and 2 females ranging from 12 to 67 years of age (median 42 years). All 8 cases involved the extremities specifically the deep soft tissues of the fingers, hand, forearm, foot or limb girdle with concomitant bone involvement in 6 cases; several appeared to be periosteal. The histologic appearance was uniform between cases & distinctive. All tumors had a lobulated growth pattern with a prominent chondroid to focally osseous matrix. Constituent cells, arranged in clusters or singly, were epithelioid with abundant eosinophilic to clear cytoplasm and variable nuclear pleomorphism. Focal necrosis was noted in 1 case. None demonstrated clear ducts, prominent spindle cells or plasmacytoid cells. Immunohistochemical stains showed 5/6 cases were positive for pancytokeratin, 4/6 for S-100 protein, 2/4 for p63 and 5/5 for SOX-9. 2 patients had metastasis: 1 to regional lymph nodes & the other to lung.
Conclusions: 1) Parachordoma involving soft tissue & bone has a striking predilection for the extremities & frequently involves the bone and soft tissue simultaneously. 2) It is locally aggressive & may metastasize. 3) It is histologically similar to chordoma. 4) The histologic differential diagnosis includes myoepithelioma, chondroid lipoma, extraskeletal myxoid chondrosarcoma, ossifying fibromyxoid tumor of soft parts, matrix producing breast carcinoma & metastatic chordoma. 5) The majority of parachordomas express keratin and S-100 protein as is seen in axial chordoma. It also demonstrates nuclear expression of SOX-9. 6) Clinical & histological features of parachordoma lend strong support to the concept that "parachordoma", as defined in this study, is closely related to notochordal derived tumors (axial chordoma). 7) The intimate association of parachordomas with bone and soft tissue raises the possibility that periosteum/synovium may be the site of origin for this tumor.

105 Osteochondroma Formation: Haploinsufficiency or Two Hits?

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Background: Multiple osteochondromas (MO) is an autosomal dominant disorder caused by germline mutations in EXT1 and/or EXT2, whereas solitary osteochondroma is a non-hereditary lesion. EXT is involved in heparan sulfate biosynthesis. We investigated the controversial issue whether osteochondromas arise via the classical two-hit model for tumor suppressor genes or via haploinsufficiency.
Design: An *in vitro* 3D chondrogenic pellet model was used to compare heterozygous mesenchymal stem cells (MSCs)($EXT^{wt/-}$) of MO patients with normal MSCs and the corresponding tumor specimens (presumed $EXT^{-/-}$). EXT mutations and mRNA expression levels were assessed. HS chain length and structure of normal and heterozygous MSCs in monolayer culture was determined. Immunohistochemistry was performed on heparan sulfate (HS), heparan sulfate proteoglycans (HSPGs)(SDC2-4, perlecan, CD44v3), and HS dependent signaling pathways: TGFbeta/BMP (phosphosmad-1, phosphosmad-2, PAI-1), Wnt (beta-catenin) and PTHLH (PTHR1, bcl2).
Results: Germline EXT1 and EXT2 mutations were present in MO patients (6/8). We demonstrated a second hit in the EXT genes in 5 out of 8 osteochondromas (both solitary and hereditary). MSCs with a heterozygous EXT mutation are identical to wildtype MSCs with regard to HS chain length and structure, *in vitro* chondrogenesis and the expression of EXT and EXT downstream signaling molecules.
Conclusions: In conclusion, since I) a heterozygous EXT mutation does not affect chondrogenesis, heparan sulfate and downstream signaling pathways and II) we show a second hit in the majority of osteochondromas our results refute the haploinsufficiency theory and strongly support the two-hit model for osteochondroma formation.

106 Low Grade Fibromyxoid Sarcoma and Sclerosing Epithelioid Fibrosarcoma – A Clinicopathological Study of 15 Cases, Including Identification of 'Kinship' between These Two Sarcomas

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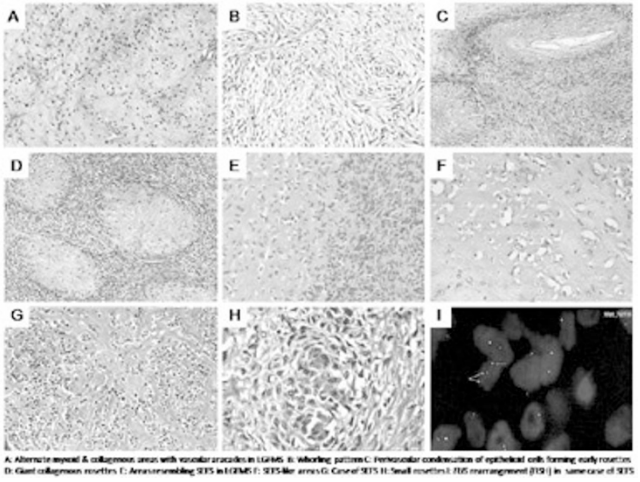
Background: A morphological and lately, genetic resemblance in form of *FUS* rearrangements, has been identified in low grade fibromyxoid sarcoma (LGFMS) and hyalinizing spindle cell tumor with giant rosettes (HSCTGR). Few studies have also noted resemblance between LGFMS and sclerosing epithelioid fibrosarcoma (SEFS).
Design: We critically analyzed histomorphological features of 13 LGFMS cases and 2 SEFS cases, over 7 years, including identification of morphological similarity between these rare sarcomas. Two SEFS cases underwent FISH analysis for *FUS* rearrangement.
Results: Thirteen LGFMS cases, identified in 7 women and 6 men, had age range of 10-50 years (median =31 years), tumor (T) size varying from 2-16.5 cm (mean = 5.8 cms) and commonly occured in lower limb (6 cases). All cases showed variable, alternating myxoid & collagenous areas with spindle to polygonal cells and vascular arcades (11 cases). Mild (10 cases) to moderate atypia (3 cases); mitosis varying from nil (maximum cases) to 3/10hpf (1 case), intranuclear inclusions (5 cases), focal hyalinization (6 cases), perivascular hyalinization (6 cases), small / 'early' rosettes (6 cases), amianthoid fibres (3 cases) were noted. Four cases with giant collagenous rosettes were labeled as HSCTGR. **SEFS-like areas were focally noted in 5 cases.** On IHC, vimentin was consistently positive in all cases. MIB-1 ranged from 1-8% and was low in maximum cases. Three of 5 cases with wide excision have been free of disease (FOD) over 5-46 months. Other 7 excisions with unclear margins included 1 FOD, 1 metastatic & 1 recurrent case. Two SEFS cases occurred in thigh of 12 & 53 year old patients. The first case underwent wide excision for a 3.5 cm tumor; displayed focal vascular arcades and small rosettes, resembling LGFMS, in addition to 'classical' SEFS and was positive for *FUS* rearrangement. The other case was negative for *FUS* rearrangement. Both cases were of low-grade, showed mild atypia, no necrosis and low MIB-1 (1-2%).



Conclusions: Morphologic 'kinship' exists between LGFMS and SEFS. It is suggested that at least some SEFS cases form spectrum of LGFMS. Larger studies need to further validate this observation.

107 Heterogeneous and Complex Rearragements of the Long Arm of Chromosome 6 in Chondromyxoid Fibroma

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Background: Chondromyxoid fibroma (CMF) is a benign cartilaginous tumour of bone mainly occurring in the second decade of life. Recurrent chromosomal rearrangements of chromosome bands 6p23-25, 6q12-15 and 6q23-27 have been reported in these tumors. The aim of the study is to further understand the role of these regions in the pathogenesis of CMF.

Design: We collected 42 CMFs and used a panel of molecular cytogenetic tests verified by immunohistochemical staining to investigate the implicated regions of interest . Whole genome copy number screening was performed by array-CGH in 15 cases. Three candidate regions (6q13, 6q23.3 and 6q24) were investigated in detail by FISH probe sets bracketing fine mapped breakpoint regions in 29 cases and the potential diagnostic relevance of any of these regions was analyzed. The expression level of nearby candidate genes was evaluated by immunohistochemistry and Q-RT-PCR in 25 and 26 cases, respectively.

Results: Fourteen of twenty-one cytogenetically analyzed cases had structural aberrations involving chromosome 6. Seventeen of twenty-nine cases (58%) showed no rearrangement at FISH analysis of the breakpoint regions. A common minimal region of deletion was detected in three cases at 6q24 by array-CGH. The hemizygous deletion in 6q24 was verified in all three cases and an additional case was identified. The other two investigated regions showed both balanced as well as unbalanced rearrangements of 6q13 and 6q23.3 in six and five cases, respectively. Expression profiling of the two known tumour suppressor genes (*PLAGL1* and *UTRN*) residing at 6q24 and the *BCLAF1*, at 6q23.3, showed no changes.

Conclusions: We identified recurrent balanced and unbalanced rearrangements of three bands on chromosome arm 6q in CMF: 6q13, 6q23.3, and 6q24. Based on these results we could conclude that genetic alterations in CMF are heterogeneous; the observed changes may be secondary to either a cryptic translocation or a point mutation. All together rearrangements of these three regions occur in less than half of CMFs, reducing their diagnostic usefulness.

108 DOG1 Rescues Most CD117-Negative GISTs: A Report from an Italian Multi-Institutional Study (REGISTER Study)

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Background: DOG1 is an emerging highly specific and sensitive immunohistochemical marker of GISTs. The aim of our study was to confirm the diagnostic utility of this antibody in a large Italian multi-institutional GISTs series (REGISTER) and in particular its ability to discriminate GIST from GISTs mimics.

Design: Immunohistochemistry for DOG1 (1:400, WB pH6, clone SP31, ACZON) was performed on 42 tissue microarray (TMA) slides, which included 583 CD117-positive GISTs and 45 CD117-negative GISTs. Furthermore 4 TMA slides containing 73 mesenchymal tumours of the GI tract, including 21 leiomyomas, 16 leiomyosarcomas, 12 pleomorphic sarcomas and 10 desmoid fibromatoses, were also immunostained.

Results: DOG1 immunostaining was positive in 574 (98.5%) of CD117-positive GISTs and in 37 (82.2%) of the CD117-negative GISTs. The majority of the positive cases (96.2%) showed a diffuse and strong staining (Table 1). Of the 73 GISTs mimics, only 2 smooth muscle tumors proved to be DOG1 positive (Table 2).

Conclusions: DOG1 is expressed in most of CD117-negative GISTs, indicating its higher sensitivity as GIST marker. As a small subgroup of CD117-positive GISTs failed to stain with DOG1, the combination of CD117 and DOG1 is highly recommended for the diagnosis of GIST in daily practice.

Table 2: DOG1 expression in GISTs mimics

N of cases	histotype	DOG1 +ve, N (%)	DOG1 -ve, N (%)
21	leiomyomas	1 (4.8)	20 (95.2)
16	leiomyosarcomas	1 (6.2)	15 (93.8)
12	pleomorphic sarcomas NOS	0	12 (100)
10	desmoid fibromatoses	0	10 (100)
4	spindle cell sarcomas	0	4 (100)
3	schwannomas	0	3 (100)
6	others	0	6 (100)

Table 1. DOG1 expression in CD117-positive and CD117-negative GISTs

	N of GISTs cases	DOG1 +ve		DOG1 -ve
		strong/diffuse	weak/focal	
CD117 +ve GISTs	583	555 (95.2%)	19 (3.3%)	9 (1.5%)
CD117 -ve GISTs	45	33 (73.3%)	4 (8.9%)	8 (17.8%)

109 IgG4 Plasma Cells in Inflammatory Myofibroblastic Tumor: Inflammatory Marker or Pathogenic Link?

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Background: Inflammatory myofibroblastic tumor (IMT) harbors a plasma cell rich inflammatory infiltrate. In contrast, IgG4 related sclerosing disease (IgG4SD) encompasses a syndrome with multisystem involvement by a fibroinflammatory proliferation with abundant lymphoid aggregates (LA) and obliterative phlebitis (OP). Cellular IgG4SD overlaps histologically with IMT. We investigated clinicopathologic and immunohistochemical features with IgG4 plasma cells in 36 IMTs.

Design: IMTs were retrieved from consultation and institutional files. Pathology materials and medical records were reviewed. Diagnosis was based on WHO criteria. Immunohistochemical stains on formalin-fixed paraffin-embedded tissue included ALK, smooth muscle actin, IgG, IgG4, CD35, CD21, and CD23. IgG and IgG4 positive plasma cells were counted in 6 40x HPF, with calculation of the IgG4/IgG ratio.

Results: 36 IMTs (ages 1-41 years, 92% in first 3 decades; 19 females, 17 males), originated in the mesentery (47%), lung (19%), urinary bladder (11%), pelvis/peritoneum (14%), or elsewhere (8%).Diameter ranged from 2-41 cm. None had OP or abundant LA. 64% were reactive for ALK. Mean IgG4 plasma cells per HPF ranged from 0-33. 16 IMTs (44%) had an IgG4/ IgG ratio>0.10 with no significant difference between ALK positive and negative cases. The IgG4/IgG ratio ranged from 0.1 to 3.24. 63%were ALK positive, 47% were CD35 positive (all negative for CD21 and CD23), 37% had the inflammatory clinical and laboratory syndrome associated with IMT, and 17% had recurrence.

Conclusions: IMTs have distinctive histology, lack OP and prominent LA of IgG4SD, and have fewer IgG4 plasma cells than IgG4SD. Although the number of IgG4 positive plasma cells in IMT is lower than published ranges for IgG4SD (33 vs. 60-100 per HPF), the IgG4/IgG ratio in 44% of IMTs in this series overlapped with the published cutoff of > 0.10 for IgG4SD. There was no significant difference in IgG4 expression between ALK positive and ALK negative IMTs. CD35 reactivity may reflect complement involvement in the inflammatory process. We conclude that IgG4 plasma cell counts and IgG4/IgG ratios are not reliable diagnostic discriminators. Clinical and histologic features, presence or absence of obliterative phlebitis and use of diagnostic adjuncts as ALK immunohistochemistry allow recognition of IMT, which has clinical and therapeutic significance. The findings in this study do not support the hypothesis that IMT is a variant of IgG4SD.

110 Schwannoma of Bone: A Clinicopathologic and Radiologic Study

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Background: Schwannoma of bone is a rare neoplasm occuring most commonly in the jaws,temporal bone and sacrum. Although apparently arising in bone, they actually arise from nerves traversing bone within intraosseous foramina. Herein, we characterize the clinicopathologic features of 17 schwannomas originating within the substance of bone and radiologically mimicking other, more frequent primary bone tumors.

Design: Of 217 schwannomas involving bone and either operated at our Institution or seen in consultation between the years of 1974 to 2004, 17 were apparently primary in bone. Tumors excluded were a) situated in soft tissue and causing secondary bone erosion or b) within nerve foramina of the jaws, temporal bone or sacrum, and c) lesions histologically reclassified. Immunohistochemical studies directed against S-100 protein, collagen IV, HMB-45, Melan A and Ki-67 antigen were performed.

Results: In our series, schwannomas of bone represented 1% of primary bone tumors seen over a 30 year-period. Median patient age was 40 yrs (range, 9-56), with a slight female predilection (F:M=1:0.7). Long bones (71%), particularly the diaphysial region, were most often affected. Pain was the most frequent complaint. Of the 17 lesions, 13 were conventional schwannomas, two with degenerative atypia (ancient schwannoma), 1 was cellular and 3 were melanotic, 2 being psammomatous. Aside from the 2 psammomatous melanotic schwannomas that occurred in Carney complex, the remaining lesions were solitary and sporadic having no association with neurofibromatosis or schwannomatosis. Mitoses ranged from 0 to 2 /10HPF. No necrosis was observed. Immunostaining included S-100 protein and pericellular collagen IV in all tumors tested. MIB1 index was uniformly low. All melanotic tumors were also immunoreactive

for HMB45 and Melan A. Follow-up information was available in 11 cases (64%) and ranged from 4 to 19 yrs. One conventional schwanoma recurred at 4 yrs; the patient is tumor-free at 9 yrs. No patient developed metastases or died of disease.
Conclusions: As schwannoma of bone is rare, it is often not included in the differential diagnosis of primary osseous spindle cell tumors. Awareness of its occurrence, particularly of the cellular and melanotic variants should preclude histologic misdiagnosis and overtreatment. Like their far more common extraosseous counterparts, schwannoma of bone behaves in a benign fashion and is successfully treated by local excision alone.

111 Tenosynovitis with Psammomatous Calcification: A Poorly Recognized Pseudotumor Related to Repetitive Tendinous Injury

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Background: The terms "idiopathic calcifying tenosynovitis" and "calcific tendinitis" are clinical and radiographic terms used to describe a syndrome of pain and tendinous calcification, most often involving the distal supraspinatus tendon of the shoulder region. The histopathological correlates of this clinico-radiographic syndrome were initially described in 1983 in a series of 2 cases by Gravanis and Gaffney, who coined the term "tenosynovitis with psammomatous calcification" to describe their morphologic findings. Subsequently only a very small number of similar cases have been reported, including some apparently included in a recent large series of mixed calcareous lesions of the distal extremities. For these reasons the morphological features of this entity remain poorly appreciated by pathologists, judging from our recent consultation experience.
Design: Five cases meeting criteria for tenosynovitis with psammomatous calcification were retrieved from our consultation and institutional archives. Clinical and follow-up information was obtained from the referring pathologists and our medical record system. All available hematoxylin and eosin-stained slides were reviewed.
Results: Cases involved the tendons and adjacent synovium of the distal extremities (2 feet, 1 carpal tendon, and 2 fingers) of women <20 (2 cases), 40 (1 case) and >60 92 cases) years of age. The lesions presented as a painful mass; a history of occupational or sports-related repetitive motion and/or persistent mild trauma was noted in all but one patients. No patient had a history of hyperphosphatemia. All lesions were treated by surgical excision and described clinically as variably cystic nodules composed of amorphous "cheese-like" debris. Histologically, the lesions were centered in the tendon and composed of a mixed (myo)fibroblastic and histiocytic proliferation in association with dystrophic calcification, including distinctive psammoma body-like spheroidal bodies.
Conclusions: The clinical and morphological characteristics of tenosynovitis with psammomatous calcification (distal location, absent hyperphosphatemia, psammatous calcifications) differ from those of typical idiopathic calcifying tenosynovitis/ calcific tenditinitis (proximal location, dystrophic tendinous calcification) and tumoral calcinosis (hyperphosphatemia, amorphous soft tissue calcification), and it should be recognized as a distinct clinicopathological entity. Improved recognition of these unique features by pathologists should allow ready diagnosis of this unusual pseudotumor in most instances.

112 Dedifferentiated Liposarcoma with Inflammatory Myofibroblastic Differentiation

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Background: Dedifferentiated liposarcoma (DL) is defined by juxtaposition of well differentiated liposarcoma (WDL) and non-lipogenic sarcoma (NLS). In most tumors the NLS resembles pleomorphic undifferentiated sarcoma or myxofibrosarcoma. However, various patterns and heterologous lines of differentiation have been described. We have observed cases of DL with prominent myofibroblastic features associated with chronic inflammation that closely resemble inflammatory myofibroblastic tumor (IMT). Such tumors can potentially be misdiagnosed as IMT. To our knowledge this pattern has not been previously reported, nor has its morphologic, immunohistochemical and clinical spectrum.
Design: Among 35 DL diagnosed at our institution between 1990-2009, 6 had prominent IMT-like features. For each tumor we tabulated the extent of IMT-like histology (diffuse or focal), the subtype of WDL, and presence or absence of atypical cytological features within the IMT-like areas. Whole block sections were immunostained for IMT-associated markers (SMA, desmin, ALK-1) and liposarcoma-associated markers (MDM2, S-100).
Results: Mean age was 62 years (range 41-77) with a 5:1 male:female ratio. 2 (33%) were located in the inguinal/scrotal region, 2 (33%) in the retroperitoneum, 1 (17%) involved both inguinal/scrotal and retroperitoneum, and 1 (17%) was intraabdominal. Average tumor size was 19 cm (range 11-28). 4 (67%) were high-grade and 2 (33%) were low-grade. In 4 tumors (67%) the NLS component dominated, while in 2 it was a minor component. 2 tumors had diffuse IMT-like areas within the NLS component, while in 4 it was focal. 2 tumors had numerous ganglion-like fibroblasts, 2 had fibromatosis-like areas, 1 had osseous metaplasia, and 1 had a prominent nodular fasciitis-like appearance. 4 had atypical cells within the IMT-like areas. In 5 tumors (83%), the WDL was lipoma-like, while in 1 (17%) it was inflammatory WDL. SMA was positive in 4 tumors (67%), desmin in 4 (67%) and MDM2 in 2 (33%). No tumor showed ALK-1 or S-100 expression. Mean follow-up was 26 months (range 2-66). 3 patients had documented metastases, 3 had local recurrences, 2 died of disease, 2 were alive with disease, and 2 were alive without evidence of disease.
Conclusions: DL may have prominent myofibroblastic differentiation including areas indistinguishable from IMT, which may lead to misdiagnosis. These tumors have a predilection for the inguinal/scrotal region and retroperitoneum, are more common in men, and like conventional DL may show aggressive behavior.

113 Solitary Fibrous Tumor of the Retroperitoneum and Abdomen/ Pelvis: A Clinicopathological, Immunhistochemical and Cytogenetic Analysis of 29 Cases

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Background: Solitary fibrous tumor of the retroperitoneum and abdomen/pelvis (SFT-RAP) represents 9-24% of extrathoracic SFTs. SFT-RAPs comprise a large proportion of reported cases with atypical/malignant features. We performed a clinicopathological, immunohistochemical and cytogenetic analysis of a large series of SFT-RAPs.
Design: Twenty-nine cases of SFT-RAP were retrieved from our files. Cases were examined for size, growth pattern, cytological atypia, cellularity, mitotic activity, necrosis, and unusual features. Immunohistochemical stains were performed using the avidin-biotin-peroxidase complex technique. Follow-up information was obtained from the medical record.
Results: Patients ranged in age from 25-79 (ave=57) years. 62% were female and 38% male. 12 cases arose in the retroperitoneum and 17 in the abdomen/pelvis. Grossly, all tumors were solitary and circumscribed except for 2 cases that had infiltrative margins and 1 that presented as multiple mesenteric nodules. Average tumor size was 12.1 (range 5-25) cm. 64% of cases measured greater than 10cm. Myxoid change was present in 35% of cases (5-95% of tumor). Lipomatous metaplasia was seen in 3 cases. Epithelioid cytology was evident in 4 cases. Marked stromal or perivascular hyalinization was identified in 7 cases. Striking amianthoid fibers were present in 1 case. 17 cases (59%) had at least one atypical histological feature (atypia, increased cellularity, >4 mitoses/10hpf, or necrosis). 25/28 cases were positive for CD34, 11/11 positive for CD99, and 3/4 positive for bcl-2. Rare cases showed focal staining for actin, desmin, S100, EMA, or pancytokeratin. Cytogenetic aberrations, including four cases with translocations, two of which involved 18p11.2, were identified in 5/9 cases studied. Follow-up ranging from 2-120 (ave=38) months was available in 22 cases. Seven patients developed local recurrence and/or metastasis. Six of these had atypical histological features of which 5 had increased mitoses and/or necrosis.
Conclusions: SFT-RAPs commonly have atypical/malignant histological features. Other unusual features including myxoid change, lipomatous metaplasia, hyalinization, and epithelioid cytology may be present. Cytogentic aberrations may include translocations involving 18p11.2. Similar to SFTs at other sites, the presence of atypical/malignant features is not necessarily predictive of behavior. However, tumors with increased mitoses and/or necrosis often behaved aggressively.

114 Chondroid Tenosynovial Giant Cell Tumor of the Temporomandibular Joint: Clinicopathologic and Immunohistochemical Analysis of 5 New Cases

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Background: Chondroid metaplasia in tenosynovial giant cell tumor (TGCT) is rare with only 4 well documented cases reported in the literature, 2 of which involved the temporomandibular joint (TMJ). We report 5 new cases of chondroid TGCT all arising in the TMJ. The clinicopathological and immunohistochemical features and distinction from other chondroid lesions involving the TMJ are discussed.
Design: Five cases of TGCT with areas of chondroid metaplasia involving the TMJ were retrieved from our files. Routine H&E sections were reviewed for the extent of chondroid metaplasia, patterns of matrix formation and mineralization, cellular composition of conventional TGCT within the tumor, architecture, and bone invasion. Immunohistochemical stains for Clusterin, D2-40, Desmin, CD163 and S100 protein were performed using an ABC method. Follow-up information was obtained from the medical record.
Results: Three patients were male and two female. Age ranged from 36-70 (mean=49) yrs. Average tumor size was 3.9 (3.1-4.5) cm. Grossly, tumors had a red-brown or grey-white appearance depending on the extent of chondroid metaplasia present. All cases were nodular and grew with a pushing front without bone invasion. Chondroid metaplasia had a geographic or lobular architecture, was extensive in three cases comprising over 90% of the tumor, and was less extensive (30%) in the other two. The matrix ranged from poorly formed and myxoid with lace-like calcification to better developed hyaline-like cartilage with more diffuse calcification. The conventional TGCT component contained multinucleated giant cells, large mononuclear synovial cells, and smaller histiocytic cells containing hemosiderin. Staining for Clusterin and D2-40 was seen in the large cells in both the chondroid and conventional components in all 5 cases. Large cells were focally positive for Desmin in 2 of 5 cases. CD163 highlighted a population of smaller histiocytes in 4 of 5 cases. Variable staining for S100 in chondroid areas was seen in 3 of 5 cases. Follow-up ranging from 8 to 67 (ave=29) months was available in all cases . One patient had recurrent disease 3 years after an initial excision.
Conclusions: Chondroid TGCT is a rare, distinct synovial tumor with a predilection for the TMJ that needs to be distinguished from other chondroid lesions, including chondroblastoma and chondrosarcoma, in this region. Chondroid metaplasia may be extensive. Recurrences do occur, but seem to be uncommon.

115 Podoplanin (D2-40) Expression in Mesenchymal and Clear Cell Chondrosarcoma

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Background: Cartilaginous tumors include a variety of benign and malignant entities which may present diagnostic challenges on histopathology. For example, mesenchymal chondrosarcoma (MC) can be hard to differentiate from other small blue cell tumors especially when it lacks chondroid matrix. Similarly, chondroblastoma (CB) and giant cell tumor (GCT) are both epiphyseal tumors with frequent giant cells which may have overlapping features. Podoplanin (D2-40), widely known as a lymphatic endothelium marker, is also expressed in cartilage, chondromas and some conventional chondrosarcomas. However, D2-40 expression in other cartilaginous tumors, including mesenchymal and clear cell chondrosarcoma (CCC), has not been investigated in the

literature. SOX-9 is a master regulator for chondrogenesis, and has been suggested to be a useful diagnostic marker for mesenchymal chondrosarcoma. Our goal is to study the expression patterns of D2-40 and SOX-9, and assess their potential diagnostic values in several cartilaginous tumors.

Design: With IRB approval, 7 MC, 4 CCC, 12 CB, 11 GCT, and 12 aneurysmal bone cysts (ABC) were selected from University of Chicago pathology archive. Representative sections were stained for D2-40 and SOX-9 by standard immunohistochemistry. The staining intensity and percentage of positive cells were recorded, and graded as: diffuse (>50%), focal (1-50%), and negative. Normal cartilage and lymphatic vessels were excluded from evaluation.

Results: The results are summarized in Table 1. In positive MC cases, D2-40 and SOX-9 were strong, both in chondroid foci and in undifferentiated areas composed entirely of small blue cells. Interestingly, the single D2-40 negative MC had pleomorphic morphology and only focal SOX-9 staining. Both markers showed strong staining in CCC. CB had significantly stronger and more diffuse D2-40 and SOX-9 staining than GCT and ABC (all p values < 0.01, Mann Whitney U test). Most D2-40 positive GCT cases only had scattered cells (< 5% of total cells) highlighted by this marker.

Table 1.

Cases	D2-40			Sox9		
	Diffuse	Focal	Negative	Diffuse	Focal	Negative
MC	6	0	1	6	1	0
CCC	4	0	0	3	1	0
CB	8	4	0	7	2	3
GCT	0	7	4	0	4	7
ABC	0	0	12	0	7	5

Conclusions: D2-40 could be a useful diagnostic marker for MC, CCC and CB, either used alone or combined with SOX-9. The diffuse, strong D2-40 staining in the undifferentiated small blue cell areas of MC can be particularly useful. This study is the first to characterize D2-40 expression in MC and CCC, and SOX-9 expression in CCC.

116 Primary Cutaneous Epithelioid Angiosarcoma: A Clinicopathologic Study of 13 Cases

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Background: Epithelioid angiosarcomas (EAS) are rare aggressive neoplasms, most frequent in deep soft tissues. Visceral / cutaneous lesions are reported. Primary cutaneous lesions are rare, with discrepancy in the literature regarding their behaviour. Some authors suggest they may be less aggressive than their soft tissue counterparts. We report a series of 13 cases of cutaneous EAS, and analyse their clinicopathologic features.

Design: 19 cases of EAS were retrieved from our referral archives. Lesions on the head and neck of elderly patients, post-radiotherapy, in association with lymphoedema, or deemed to be metastatic / extension from soft tissues were excluded. Thirteen cases of primary cutaneous EAS were examined for histopathological and immunohistochemical features. Clinical details, treatment and follow-up were requested from referring pathologists in all cases.

Results: Primary cutaneous epithelioid angiosarcoma occurred in adults (mean age 66 years) with an equal sex distribution, and presented as solitary (n=11) or multiple (n=2) nodules ranging in size from 8 – 80 mm, with predilection for the limbs (n=10), Histopathologically the lesions comprised infiltrative sheets of atypical epithelioid cells within the dermis +/- subcutis. 5 lesions were at least focally polypoid. Epidermal hyperplasia was noted in 5 cases, and ulceration in 4. Vascular channel formation and intracytoplasmic lumina were seen at least focally in 11 and 10 cases respectively. Mitoses were readily identified and necrosis was seen in 5 cases. The neoplasm was immunoreactive for the vascular markers CD31 and FLI-1 in all cases, and CD34 in half of the cases tested. Pancytokeratin (MNF116) was positive in two-thirds of cases, and EMA in a quarter of cases. There was rare focal expression of SMA. S100 protein, HMB45, desmin, CD30 and HHV8 were negative, whilst INI1 was positive in all cases tested. Follow-up information was available for 11 patients. 7 patients had staging CT scans, 5 showing evidence of dissemination, with regional lymph node involvement (n=1), liver (n=1), lung (n=1), bones (n=1) and regional lymph node + liver (n=1). Treatment was surgical +/- adjuvant radiotherapy in all cases. 6 patients died from metastatic disease after a median follow-up of 12 months (3-36 months), and 1 from unrelated causes.

Conclusions: Primary cutaneous epithelioid angiosarcoma is a highly aggressive malignant tumour with mortality rates in excess of 55% after 3 years.

117 Influenza Virus Induces Apoptosis in Osteosarcoma Cells

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Background: Osteosarcoma (OS) is the most frequent primary malignant bone tumor in young adults and adolescents. Despite recent advances in multimodality treatment comprising aggressive adjuvant chemotherapy and wide local excision, pulmonary metastasis occurs in approximately 30% of patients with OS and remains a major cause of fatal outcome. Among the number of naturally occurring viruses being investigated as oncolytic agents for cancer treatment, a strain of influenza virus has been reported to induce apoptosis in numerous cell types. In this study, we analyzed susceptibility of human OS cell lines to apoptosis after influenza virus infection *in vitro*.

Design: Three OS cell lines MG63, HOS and Saos2 were infected with influenza A/Aichi/2/68 (Aichi) virus. We assessed the susceptibility of OS cells to Aichi virus by plaque assay and the growth of OS cells infected with Aichi virus by MTT assay. To detect apoptotic cells after infection with Aichi virus, TUNEL assay was performed and we examined caspase-3 activation by Western blotting analysis.

Results: Aichi virus replicated in MG63, HOS and Saos2, and formed plaques on MDCK cell monolayers. Aichi virus induced cytopathic effects in MG63 cells at 48 hours after viral infection. The viability of MG63 cells infected with Aichi virus was reduced more significantly at a multiplicity of infection (MOI) of 10 when compared to MOIs of 1 and 5. TUNEL-positive MG63 cells were observed at 48 hours after Aichi virus infection and were significantly more numerous at an MOI of 10 when compared to MOIs of 1 and 5. Western blotting analysis showed that protein levels of caspase-3 (35 kDa) were lower, and cleaved caspase-3 (19 and 17 kDa) was observed in MG63 cells infected with Aichi virus at an MOI of 10.

Conclusions: We confirmed the susceptibility of human OS cells to apoptosis induction by Influenza A/Aichi/2/68. To date, the influenza A virus has been explored as an oncolytic virus. The NS1 protein is a virulence factor that counteracts the PKR-mediated antiviral response of the host. As a consequence, influenza virus lacking the NS1 open reading frame fails to replicate in normal cells, but produces infectious particles in PKR-deficient cells. The oncolytic properties of this mutant virus are dependent on activated *Ras*, as oncogenic *Ras* induces an inhibitor of PKR. It may be possible to use the influenza virus as an agent for oncolytic virotherapy in OS.

118 Prognostic Analysis of Protein Expression by Tissue Microarray in Osteosarcoma

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Background: Osteosarcoma (OS) is the most frequent primary malignant bone tumor in young adults and adolescents. Novel markers of lung metastasis and prognosis of OS have not been reported. The purpose of this study was to determine the expression and prognostic significance of proliferating cell nuclear antigen (PCNA), p-Smad2 as a downstream signal of TGF-b, vascular endothelial growth factor (VEGF) and podoplanin in OS.

Design: We constructed a tissue microarray (TMA) block composed of 23 cases, including samples from 21 biopsies, 23 surgical specimens, 5 lung metastases and 6 bone metastases. Immunohistochemical (IHC) staining was performed with four kinds of proteins: PCNA; p-Smad2; VEGF; and podoplanin. IHC score was determined as the sum of the distribution score (0, 1, or 2) and intensity score (0, 1, 2, or 3) of the staining signal. We assessed: 1) the relationship with IHC score and overall survival; 2) the difference in IHC score between primary and metastatic sites; 3) correlations with each IHC score for the four kinds of proteins in primary site using the Spearman's rank coefficient.

Results: 1) Mean and median durations of overall patient follow-up were 75.35 and 35 months, respectively (range, 0.15-300 months). The correlation between PCNA and survival rate was significant (log-rank 0.032). Overall survival rate was 23.5% for strongly PCNA-positive cases, compared with 100% for weakly PCNA-positive cases. 2) IHC scores for PCNA, VEGF and podoplanin were significantly higher at the metastatic site than at the primary site ($p<0.01$ each). Conversely, IHC score for p-Smad2 was higher at the primary site than metastatic site ($p<0.01$). 3) Significant correlations were seen between PCNA and podoplanin ($p=0.039$, $r=0.440$), p-Smad2 and podoplanin ($p=0.007$, $r=0.568$), p-Smad2 and VEGF ($p=0.018$, $r=0.504$) and between VEGF and podoplanin ($p=0.040$, $r=0.438$).

Conclusions: 1) Our survival analysis indicated that positive PCNA staining correlates significantly with prognosis in OS. PCNA is useful as a prognostic marker for OS. 2) Podoplanin was significantly expressed at metastatic sites, suggesting that podoplanin promotes hematogenous metastasis. 3) Expression of podoplanin is correlated with expression of PCNA, VEGF and p-Smad2, respectively. As a result, podoplanin may play a significant role in lung metastasis in cooperation with VEGF and TGF-b.

119 INI-1 Expression in Cytokeratin-Positive Tumors of Bone

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Background: Loss of INI-1 expression by immunohistochemistry has been reported in rhabdoid tumors of kidney, soft tissue and central nervous system, and more recently in epithelioid sarcoma (ES) of classic and proximal type. ES is a rare soft tissue sarcoma showing epithelial differentiation, as detected by cytokeratin expression (CK), and is associated with an ominous prognosis. Recently, a case of ES presumably arising in bone has been described, a diagnosis strengthened by loss of INI-1 expression by immunohistochemistry and FISH. This tumor was characterised by a prominent matrix deposition with a chicken-wire pattern, resembling an osteosarcoma (OS) or a chondroblastoma, tumors on which INI-1 expression has not yet been investigated. Furthermore, CK expression has been described in various bone tumors including OS. In this study, we have assessed INI-1 expression on a large number of bone tumors which potentially may be included in the differential diagnosis of ES.

Design: Material included 447 bone tumors known to express CK to some extent, and comprise 276 OS, 126 chordomas, 21 chondroblastomas, 10 CK-positive PNET/Ewing's sarcomas, 7 CK-positive bone sarcoma NOS, 4 metastatic carcinoma of unknown primary (CUP), 1 malignant mixed tumors of bone and 2 epithelioid angiosarcoma of bone. OS, chordomas and chondroblastomas have been assessed on tissue microarrays; the remaining tumors on full sections. BAF47 antibody (clone 25) has been employed. A classic ES of soft tissue was used as control.

Results: Only 5 cases of 447 bone tumors showed complete loss of INI-1 expression, a finding that was confirmed on full tissue sections. These included 1CUP, 2OS and 2 chordomas (brachyury positive). Review of the H&E slides confirmed the diagnoses of OS and chordoma. The INI-1 negative CUP was found to represent a poorly differentiated vimentin and CK positive tumor with focal rhabdoid cytoplasm, which occured in the proximal femur of a 25 year-old-woman. Despite extensive investigations, this was the only lesion identified in this patient and on review is now considered to represent a possible primary ES of bone.

Conclusions: Having assessed the INI-1 expression in a large number of bone tumors, we found that less than 1% of OS failed to express INI-1 protein and report for the first time the loss of INI-1 immunoeractivity in 1.5% of chordomas. We report a potential

new case of ES of bone previously diagnosed as CUP, which prompts the assessment of INI-1 in all poorly differentiated cancers of unknown primary, especially in young individuals.

120 The Prognostic Significance of Sarcoma Metastases to Skin

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Background: Sarcoma metastases to skin are relatively rare as most metastases involve lung, liver or deeper soft tissues. Here we examine the distribution and clinical significance of cutaneous and superficial subcutaneous sarcoma metastases.

Design: 65 patients with histologically confirmed dermal and superficial subcutaneous sarcoma metastases were identified in pathology files from more than 25,000 sarcoma patients evaluated at M. D. Anderson (1989-2009). Pathology slides, clinical and radiologic information were evaluated.

Results: Cutaneous metastases were histologically documented in less than 0.25% of more than 25,000 sarcoma patients. The mean patient age was 49 years (16-79) with equivalent gender ratio. The most common source was leiomyosarcoma (28/65, 43%; see Table 1). The most common region of first skin metastasis was head and neck (33, 51%) with the scalp predominating (25,38%). Mean time from primary tumor diagnosis to skin metastasis was 48 months (0-166). 53 patients (81%) had multiple metastases (skin & other). In patients with complete clinical information available, 31 (62%) had other metastases prior to skin involvement, 17 (34%) had skin metastases first and 2 (4%) had simultaneous presentation. Clinical outcome was: 29 (45%) dead of disease; 24 (37%) alive with disease; 12 lost to follow up. Mean time to death was: (1) 80 (9-224) months from primary diagnosis; 45 (5-94) from first metastasis to any site; 27 (5-65) months from first skin metastasis.

Diagnosis of Primary Sarcomas Resulting in Skin Metastasis

Leiomyosarcoma	n=28
Epithelioid Sarcoma	n= 5
Angiosarcoma	n= 5
Undifferentiated Pleomorphic Sarcoma	n= 4
Osteosarcoma	n= 4
Alveolar Soft Part Sarcoma	n= 3
GIST	n= 2
Synovial Sarcoma	n= 2
Unclassified sarcoma	n= 2
Various (10 types, 1 each)	n=10

Conclusions: Sarcoma metastases to the skin are rare. In this large study, leiomyosarcoma was the most common source and scalp the most frequent site. The majority of patient with skin metastases has harbored previous metastases elsewhere. However, skin metastasis was the initial site in about 1/3 of cases. Thus, clinical correlation is needed before establishing a diagnosis of primary cutaneous sarcoma, particularly leiomyosarcoma of scalp. Finally, skin metastasis is usually a late event in sarcoma clinical progression and heralds a poor prognosis.

121 The Prevalence of *FUS* Rearrangement in Sclerosing Epithelioid Fibrosarcoma

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Background: Several recent reports have described low grade fibromyxoid sarcoma (LGFMS) with sclerosing epithelioid fibrosarcoma (SEF)-like areas. We recently encountered 3 such cases harboring the re-arrangement of *FUS* (16p11) characteristic of LGFMS detected by fluorescence in-situ hybridization (FISH). Since the established morphologic spectrum of LGFMS includes cases with an identical reciprocal chromosomal translocation previously described as "hyalinizing spindle cell tumor with giant rosettes", we evaluated classic cases of pure SEF for *FUS* rearrangement to determine whether this entity could be related to LGFMS as well.

Design: Available FFPE tissue from 3 genetically confirmed LGFMS with SEF-like areas and 16 classic SEF (without LGFMS areas) were retrieved from the pathology files of our institution and tabulated with clinical information. Unstained slides from FFPE blocks were prepared and FISH was performed using a commercial *FUS* break-apart probe.

Results: For the 16 SEF cases, the median age at presentation was 46 (14-78) years with equal gender ratio. SEF most commonly involved the abdomen/retroperitoneum (n=4) and lower extremities (n=4). Only 1 of 16 pure SEF cases showed rearrangement in the *FUS* locus. The 3 cases of LGFMS with SEF-like areas had *FUS* rearrangement in both the LGFMS and SEF-like areas documented by FISH.

Conclusions: Only 1 of 16 classic SEF contained a rearrangement of *FUS* (16p11) detected by FISH. Although cytogenetically-confirmed LGFMS can have SEF-like areas, *FUS* rearrangement appears to be rare in classic, pure SEF. Since *FUS* rearrangement is so characteristic of LGFMS, it seems likely that LGFMS with SEF-like areas and SEF share morphologic overlap, but SEF lacks the recurrent molecular determinate of LGFMS.

122 Ultrastructural Organisation of Cartilage in Proteoglycan-Deficient Zebrafish Mutants

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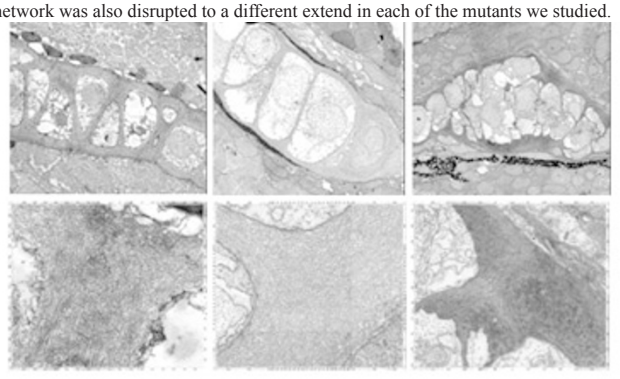
Background: Proteoglycans (PGs) are molecules consisting of a protein core onto which sugar chains, glycosaminoglycans (GAGs), are attached. PGs are produced by chondrocytes and, once secreted, they become a major component of the extracellular cell matrix (ECM). PGs and ECM are involved in many cellular events such as adhesion, migration and differentiation. Changes in PG structure and composition were found in various pathologies, e.g. osteochondromas and osteoarthritis. In order to investigate the role of different PGs in cartilage development, we studied a group of zebrafish (*Danio rerio*) mutants affected in different genes involved in PG-synthesis.

Design: We used AB (wild type) zebrafish and five mutant lines: *dak (ext2)*, which lacks Heparan sulphate only; *hi307 (β3gat3)* that is deficient in both Heparan and Chondroitin sulphates; *pic (papst1),* which is unable to sulfate PGs; *hi954 (uxs1)* that fails to initiate GAG biosynthesis and *kny (gpc4)* that does not form the protein core of Glypican 4. WT and homozygous mutants were raised till 5dpf under standard conditions, anesthetized in tricane and fixed in glutaraldehyde. Light microscopy observations were performed on five fish from each line. Single representatives of each line were also subjected to the electron microscopy.

Results: Our results show that each mutant displayed a different phenotype. For example, chondrocytes in the β*3gat3* mutant displayed a WT morphology, while the ones from the *uxs1* mutant were enlarged, misshapen and partially fused with each others (Pic. 1). However, all mutants had abnormal ECM and/or chondrocyte morphology. For instance, the β*3gat3* mutant lacked collagen network, whereas the *uxs1* mutant had bundles of short and very thick collagen fibers. The association of PG aggregates with collagen network was also disrupted to a different extend in each of the mutants we studied.



Picture 1. The organization of ECM (top) and chondrocytes (bottom) in the cartilage from wild type (left) and PG-mutants: hi307 (middle) and hi954 (right).

Conclusions: PGs have different influence on the chondrocyte morphology and the organization of the collagen network in ECM. In consequence, water-binding capacity and mechanical properties of cartilage could be altered by changes in the quality and quantity of the different PGs.

123 Establishment of a New Human Osteosarcoma Cell Line, UTOS-1: Cytogenetic Characterization by Array Comparative Genomic Hybridization

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Background: Osteosarcoma (OS) is the most common malignant bone tumor characterized by proliferation of tumor cells which produce osteoid or immature bone matrix. There have been several reports describing xenotransplantation models of human OS, but characterization of human OS at the molecular cytogenetic level has been limited. We describe the establishment and characterization of a new human OS cell line, designated as UTOS-1, derived from a conventional osteoblastic OS. In addition, we analyze chromosomal aberrations and DNA copy number changes in UTOS-1 by array comparative genomic hybridization (aCGH).

Design: Tumor cells, UTOS-1, from a typical osteoblastic OS of an 18-year-old man were cultured in RPMI 1640. To determine the tumorigenicity of the UTOS-1 cell line *in vivo*, tumor cells were injected subcutaneously into the leg of SCID mice. To determine the doubling time *in vitro*, we used MTT assay. To assess osteoblastic differentiation, we used 3 monoclonal antibodies: anti-osteopontin (OP), anti-osteocalcin (OC), and alkaline phosphatase (ALP). Expression of osteoblastic differentiation markers was also assessed by RT-PCR. For cytogenetic analysis, preparations of metaphase chromosomes from UTOS-1 cells at passage 15 were banded with Giemsa-trypsin. Array CGH was performed using the GenoSensor Array 300 system.

Results: Cultured UTOS-1 cells are spindle-shaped, and have been maintained *in vitro* for over 50 passages. The population-doubling time of the cells was 40 hours. UTOS-1 also exhibit morphological and immunohistochemical characteristics typical of osteoblastic OS. In RT-PCR, UTOS-1 cells expressed OP, OC and ALP. Chromosomal analysis by G-band showed 73-85 chromosomes with complicated translocations. Array CGH show frequent gains at locus *DAB2* at chromosome 5q13, *CCND2* at 12p13, *MDM2* at 12q14.3-q15, *FLI* and *TOP3A* at 17p11.2-p12 and *OCRL1* at Xq25, and show frequent losses at *HTR1B* at 6q13, *D6S268* at 6q16.3-q21, *SHGC17327* at 18ptel, and *STK6* at 20q13.2-q13.3.

Conclusions: We have isolated and characterized a new permanent human cell line, UTOS-1, established from an osteoblastic OS. This cell line retains the morphology, osteoblastic activities and cytogenetic characteristics of the original tumor *in vitro*. The UTOS-1 cell line is useful for biologic and molecular pathogenetic studies of human OS.

124 MDM2 and CDK4 Immunoreactivity Distinguishes Low-Grade Osteosarcoma from Benign Mimics

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Background: Parosteal osteosarcoma (POS) and low-grade central osteosarcoma (LGCOS) show similar histological appearance and clinical behavior. Recent studies have also shown that these two types of low-grade osteosarcoma (LGOS) even share a genetic background: amplified sequences of 12q13-15 including *MDM2* and *CDK4*.

The histological diagnosis of LGOS is often challenging. Because of the bland cytology and mature bony trabeculae, LGOS tends to be confused with benign lesions. POS, for example, may be mistaken as myositis ossificans (MO), osteochondroma (OC), and other surface lesions, while LGCOS may simulate fibrous dysplasia (FD) among others. The accurate diagnosis is mandated to allow appropriate management. Since benign mimics of LGOS are not expected to harbor the characteristic gene amplification of sarcoma, we reasoned MDM2 and CDK4 immunostains may aid in this difficult differential diagnosis.

Design: Twenty-two cases of LGOS from 20 patients (14 POSs, 7 LGCOSs, 1 LGOS of undetermined subtype), and 38 cases of benign histological mimics of LGOS (11 MOs, 14 FDs, 6 OCs, 1 desmoplastic fibroma, 4 florid reactive periostitides, 1 Nora's lesion, and 1 Turrett exostosis) were retrieved from the hospital files. A representative section of each case was immunostained with antibodies against MDM2 and CDK4. The results were expressed with intensity graded from 1 (weak) to 3 (strong), and with extent being either focal (1-10%) or diffuse (11-100%).

Results: Fourteen LGOSs labeled for MDM2 (14/22, 64%); and 19 cases (19/22, 86%) labeled for CDK4. All the LGOSs (22/22, 100%) expressed one or both of the markers, with 11 cases expressing both (11/22, 50%). In the majority of the cases, staining was diffuse (20/22, 91%) and in moderate or strong intensity (15/22, 68%) for either antibody. In contrast, none (0%) of 38 benign lesions demonstrated immunoreactivity for MDM2 or CDK4. The combination of these two markers thus made both sensitivity and specificity reach 100% for the diagnosis of LGOS.

Conclusions: All the LGOSs labeled for MDM2 and/or CDK4, while none of benign histological mimics expressed these markers in this analysis. MDM2 and CDK4 immunostaining may hence serve as a useful adjunct for the diagnosis of POS and LGCOS.

125 Lipomatous Neoplasms: The Diagnostic and Prognostic Implications of Molecular Classification

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Background: The use of molecular techniques has been advocated in differentiating lipomas from atypical lipomatous tumors/well-differentiated liposarcomas (ALT/WDL). However, considering that both groups of tumors can recur locally but lack metastatic potential, the practical implication of their molecular characterization has not been scrutinized. The aim of this study was to assess the clinical value of the molecular classification of lipomatous neoplasms.

Design: Four hundred five cases of lipomatous neoplasms diagnosed between 1990 and 2000 and located in the extremities were analyzed for the presence of *MDM2/CPM* amplification using fluorescence in situ hybridization (FISH). One hundred cells were analyzed in each tumor. Local recurrence-free survival was estimated with the Kaplan-Meier method and compared with the log rank test. Multivariate analysis was conducted using the Cox regression model.

Results: Using solely histologic assessment as criteria, the 405 tumors were classified as lipoma (n=324), intramuscular lipoma (n=29) and ALT/WDL (n=52). After molecular analysis, 11 of the tumors histologically classified as ALT/WDL were reclassified as lipoma (n=5) and intramuscular lipoma (n=6), whereas 7 of the tumors histologically designated as lipoma were reclassified as ALT/WDL. Follow-up information was available for 303 tumors. Prior to the molecular data, the 5-year local recurrence rates for lipoma, intramuscular lipoma and ALT/WDL were 2%, 5% and 45%, respectively (P <0.0001). After molecular reclassification, the 5-year local recurrence rates for lipoma, intramuscular lipoma and ALT/WDL were 1%, 12% and 44%, respectively (P <0.0001). Multivariate analyses showed that histologic type emerged as the only independent risk factor before molecular classification (HR_{alt}=4.15; 95% CI, 1.70-11.13; p=0.0005); however, after molecular classification both histologic subtype (HR_{alt}=2.62; 95% CI, 1.28-5.58, p<0.0001) and type of surgery (HR_{wle}=0.59; 95% CI, 0.36-0.97; p=0.036) correlated with the risk of local recurrence.

Conclusions: The use of molecular testing to complement the histologic assessment of lipomatous tumors more precisely discriminates local recurrence risks for individual groups of lipomatous tumors located in the extremities and therefore provides for appropriate surgical management decisons.

126 MicroRNA (miRNA) Microarray Analysis in Well-Differentiated (WD) and Dedifferentiated (DD) Liposarcomas (LP)

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Background: The function of a microRNA is to silence multiple target genes. Depending upon its target genes, miRNA can function to promote or suppress cell growth. Abnormal miRNA expression has been shown in various tumors including liposarcomas. We hypothesize some miRNA's are expressed differentially in WD and DD LP and that these miRNA's may be useful in identifying subtle features of dedifferentiation in a WD LP.

Design: 18 paraffin blocks with WD (n10) or DD (8) histology were retrieved from 5 WD LP and 6 DD LP. Area of WD morphology was also obtained in 4 of 6 DD LP. Total RNA was extracted from 4 x 20 µm sections for Exiqon miRNA microarrays. A pooled sample from all samples was used as common reference. Data was analyzed with SAM and Partek software to identify differentially expressed miRNAs. qRT-PCR was performed to validate selected miRNA's in the same samples.

Results: Variable numbers of human miRNA's were found to be expressed differentially between DD LP (n=6) and WD LP (n=5); tissue with DD histology (n=8) and WD histology (n=10); tissue with WD histology from DD LP (n=4) and WD LP (n=5). The down regulation of miR-539 in tissue with DD histology and miR-193a-5p in WD area from DD LP was validated by qRT-PCR.

Conclusions: The expression of specific miRNAs is different in DD LP (regardless of samples containing DD or WD histology in these tumors) compared to WD LP. Different gene expression in WD tissue from DD LP and pure WD LP has also been reported in cDNA microarray analysis.These findings suggest a role of these miRNAs in progression of LP and change in miRNA expression can occur even before or without histologic dedifferentiation. The results of this study need to be further validated in larger tumor sets. The validated results can provide guidance to identify candidate genes functioning as oncogenes or tumor suppressors in LP, and subsequently potential targets for treatment and markers for early prediction of dedifferentiation in LP.

Breast

127 Use of High Resolution Array Comparative Genomic Hybridization (aCGH) To Identify Mouse Double Minutes 4 (Mdm4) as an Early Genetic Change in Breast Cancer Development: Evaluation of Mdm4 as a New Prognostic and Predictive Marker

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Background: aCGH identified recurrent gain of chromosome 1q31-32 in >50% of BC. MDM4 gene maps to this locus. We hypothesised that it may be a candidate oncogene and tested this hypothesis on several levels: 1) Copy number alteration, 2) mRNA expression, 3) protein expression and 4) clinicopathological outcome.

Design: aCGH was performed for three independent BC series. MDM4-mRNA expression levels were assessed in 2-independent sets of gene expression arrays. Protein expression levels were assessed using immunohistochemistry in two-series of 1081 BCs with long term follow up and 140 cases of BC with matching normal terminal ductal lobular units (TDLUs) and precursor lesions.

Results: Amplification of MDM4 was detected in 15% and 8% of low and high grade BC; respectively. MDM4-mRNA expression levels significantly correlated with copy number (Pearson's correlation=0.55, p=0.0001) and this gene is overexpressed when amplified (Mann-Whitney U test p =0.0018). Mdm4 was overexpressed in 17% of BC and was associated with low grade, ER+ and normal expressions of p53, ATM and BRCA1. In cases showing coexistent precursors with invasive component, MDM4 expression was identical in both lesions. On multivariate analysis that included NPI, MDM4-overexpression was an independent prognostic marker for patients survival outcomes [HR, 0.4; p<0.0001]. In high risk ER+ patients absence of MDM4-overexpression predicted better response to hormone therapy [HR, 2.7; p<0.0001].

Conclusions: Mdm4 is an independent prognostic and predictor of BC and its overexpression could represent a novel molecular mechanism by which a subset of BC escapes p53-dependent growth control, providing new avenues for therapeutic intervention.

128 The Interaction between Mitotic Index and Bcl2 Expression Provides an Improved Separation Method for Determining Clinical Outcome Compared to Nottingham Histological Grading System (NGS)

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Background: We hypothesised that the interaction between mitotic index (M) and Bcl2 accurately discriminates between low and high-grade breast cancer (BC) and provides a more objective measure of clinical outcome than histological grade especially for patients with small size and oestrogen receptor (ER) negative cancers**.**

Design: Two independent series of 1650 and 245 invasive BC with long term follow up were subjected to immunohistochemical analysis with antibodies against apoptosis and cell cycle-related proteins. Mitotic index (M) was assessed according to Nottingham Grading System (NGS): M1: <10 mitoses; M2: 10 to 18 mitoses; M3: > 18 mitoses. Subsequently, BC were classified according to the combined M/Bcl2 profile and compared to NGS.

Results: In multivariate Cox regression models including validated prognostic factors, the subgroups defined by M/Bcl2 profile performed better than lymph node status and tumour size. Incorporation of the M/Bcl2 profile into the Nottingham Prognostic Index (NPI) accurately reclassified twice as many patients into the excellent prognosis group, improving decision-making for which patients should be spared systemic adjuvant therapy. Patients with M2-3/Bcl2- and M3/Bcl2+ (high risk) had a 2-3 fold increase in the risk of recurrence when treated with either adjuvant hormone therapy or anthracycline-based chemotherapy than those with M1/Bcl2± and M2/Bcl2+ (low risk) (HR= 3.4 (2.8-5.6); p<0.0001 and HR=2.3 (1.2-4.3); p=0.0009).

Conclusions: In conclusion a grading system defined by mitotic counting and Bcl2 expression accurately reclassified patients with NGS-G2, small size cancers or ER negative into two groups: low risk (NGS-G1 like) versus high risk (NGS-G3 like) of both BC mortality and recurrence, improving prognosis and therapeutic planning.

129 A New Morphological and Genetic Map for the Evolutionary Pathway of Low Nuclear Grade Breast Neoplasia (LNGBN) Family

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Background: There is evidence to suggest that tubular (TC) and lobular carcinoma (ILC) and their putative precursor lesions: columnar cell lesions; CCLs, low grade