32 Distribution of Lesions in Sudden Unexpected Deaths by Sarcoidosis

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Background: Sarcoidosis is a multisystem disease of uncertain etiology that may be responsible for sudden and unexpected death. There are few autopsy series of patterns of involvement of the major organs, a bias towards unexpected arrhythmic deaths; related to this is a lower rate of pulmonary involvement, even though the small group of pulmonary deaths did not show gross cardiac disease. However, in both groups, extensive involvement of other organs was typical. There is a high rate of cardiac involvement in forensic series because of a bias towards unexpected arrhythmic deaths; related to this is a lower rate of prior history of sarcoidosis in cardiac vs. pulmonary deaths.

33 NTRK1 Associated Gene Fusions in Pediatric Fibroblastic / Myofibroblastic Neoplasms: A Molecular Study of 58 Cases

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Background: The spectrum of pediatric fibroblastic and myofibroblastic soft tissue tumors includes a number of locally aggressive neoplasms with sometimes overlapping morphology, such as myofibroma/myofibroblastoma (MYO), lipofibromatosis (LPF), fibrous hamartoma of infancy (FHI), and calcifying aponeurotic fibroma, among others. As no prior study has carried out a comprehensive genetic investigation in this group of tumors, we applied the latest whole transcriptome sequencing for better molecular classification and to evaluate potential shared pathogenesis.

Design: RNA sequencing and FusionSeq analysis was performed on 9 cases (4 LPF, 1 FHI and 4 MYO) for novel fusion gene discovery. Validated fusion candidates by RT-PCR were then screened using FISH in a large cohort of cases.

Results: 58 cases were selected - 28 LPF, 11 FHI and 19 MYO. RNA sequencing identified gene fusions in 3/9 cases: 2 cases showed EWSR1-TERT fusions and 1 MYO showed TPM3-NTRK1 fusions. The 2 NTRK1-fused cases showed strong expression for NTRK1 by IHC. Further screening by FISH showed another LPF with TPM3-NTRK1 fusion, while 18 additional LPF showed recurrent complex FISH abnormalities at the lpq22-23.1 locus that includes NTRK1 and a number of known NTRK1 fusion partners in other cancers. Ten of 11 FHI cases showed recurrent abnormalities in the same lpq22-23.1 region. In contrast only 2 additional MYO cases (2/18) showed lpq22-23.1 FISH abnormalities. No additional EWSR1 gene rearrangements were identified in 7 cases tested.

Conclusions: Our results show recurrent NTRK1 related gene fusions in a subset of LPF and rare MYO lesions. The high rate of lpq22-23.1 locus FISH alterations spanning the NTRK1 gene in both LPF and FHI suggests the possibility of recurrent intrachromosomal fusions / regional abnormalities. These alterations require a higher resolution methodology for more detailed characterization.

ANNUAL MEETING ABSTRACTS

34 Primary Adult Skeletal Osteosarcoma: A Clinicopathological and Molecular Study

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Background: Osteosarcoma is the most common primary skeletal sarcoma with a bimodal age distribution occurring in adolescents (10-14 years) and older individuals above 40 years. Compared to pediatric osteosarcoma, it has been reported that adult skeletal osteosarcoma (>19 years) has a worse outcome. Herein, we investigate the clinicopathological and molecular features of a series of primary adult skeletal osteosarcomas and explore potential morphological and molecular parameters that may affect outcome.

Design: 18 cases of primary adult skeletal osteosarcomas were retrieved and reviewed. Clinical history and follow up were obtained through electronic record review. DNA from FFPE tissue was extracted and processed from 8 cases. DNA copy number alterations (CNA) and allelic imbalances (AI) were analyzed by genome-wide high-resolution SNP-array (OncoScan, Affymetrix).

Results: Our series include 18 patients (male=9, female=9) with a median age of 30 years (19-58) and a median follow up of 52 months (2 months - 19 years). Tumor morphologies were variable and included undifferentiated high grade pleomorphic sarcoma (n=8), osteoblastic (n=5), chondroblastic (n=2), giant cell rich (n=1), telangectatic (n=1) and epithelioid (n=1) subtypes. Tumors sites included axial (n=3) and extraxial head and neck (n=1) swellings in patients (28%) died of disease. Nine (50%) showed no evidence of disease while seven (39%) showed local recurrence and/or metastasis at last follow-up. Genomic analysis of osteosarcoma tumors suppressor genes deregulated in pediatric osteosarcoma showed frequent copy number alterations (gains and losses) in all 8 cases studied. Most common alterations included loss of heterozygosity, including deletion and copy number loss-LBH, of tumor suppressor genes TP53 (30%), CDKN2A (60%), RB1 (63%), PTEN (50%), CDH1 (50%) and LKAMP (63%). Homozygous deletion of CDKN2A was found in 25% of cases. In addition, amplifications or copy number gains of oncogenes were present as follows: CDC6, BUB1, CCND4 at 6p12-21 in 37% cases, MYC at 8q24 in 30% cases, MMCD and CDK4 at 12q13-15 in 37% cases, EGR4 in 25% cases.

Conclusions: 1) Our findings suggest that the primary adult skeletal osteosarcomas share many of the genetic alterations seen in pediatric osteosarcomas. 2) Follow-up showed 39% with local recurrence and/or metastatic disease and 28% with a median disease free survival of 21 months (1-84). 3) Additional cases are being studied by SNP-array analysis and targeted next generation sequencing which may aid in the detection of genetic alterations that may be specific to primary adult skeletal osteosarcomas and thus may provide insight into their clinical behavior.

35 Histologic Spectrum of Giant Cell Tumor (GCT) of Bone in Patients < 18 Years of Age: A Study of 66 Patients

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Background: While the majority of GCTs occur in adult patients, occasionally they arise in the pediatric population. In this setting they may be mistaken for tumors more commonly seen in this age group, particularly osteosarcoma due to aggressive behavior, findings and the presence of immature bone. In order to better understand how to avoid this problem, we studied a series of GCTs in patients ≤18 years of age with an emphasis on the histologic features.

Design: All cases of primary GCT of bone in patients ≤18 years were retrieved from our institutional archives. H&E slides were examined with focus on: patterns of bone formation and fibrosis, mitotic activity, necrosis, atypia and collections of foamy histiocytes. Clinical records/radiologic data were reviewed in all cases.

Results: 66 of 710 patients with histologically confirmed GCT of bone ≤18 years of age, including 45 females and 21 males (age range 8 to 18 years, median 16.5 years), were identified. Tumors involved the tibia (17), femur (14), sacrum (8), vertebral bodies (7), radius (5), humerus (4), metacarpal bone (3), fibula (2), and 1 each of the phalanx, ulna, pelvis, patella, calcaneus and navicular bone. Of cases with available imaging, 24 were epiphyseal, 9 were metaphyseal, and 5 involved both. Mature bone was present in 19 tumors (29%); 36 tumors (54%) had irregular, lace-like osteoid and 3 cases (4%) exhibited concentric whorls of osteoid. Zones of dense fibrosis, at times mimicking osteoid, were present in 28 of 66 cases (42%). The mitotic rate ranged from 1-35 mitoses/10 HPFs (median 5), necrosis was present in 12 tumors (18%), and 8 (12%) displayed collections of foamy histiocytes. None of the tumors showed cytologic atypia. Follow-up information (N=56) for 40 months, showed 19 patients with local recurrence, and 1 with benign GCT lung metastasis. The median mitotic rates for those patients without recurrence, with recurrence and with metastasis were: 5 (range 1-35), 7 (range 1-24) and 7. 50% of tumors with necrosis recurred (6/12) compared to a recurrence rate of 11% (6/54) in tumors lacking necrosis.

Conclusions: GCT arising in the pediatric population is rare, representing 9% of GCTs seen at our institution. The morphologic spectrum of these tumors is broad and similar to that seen in patients ≥18 years of age. It is important to recognize patterns of osteoid deposition and fibrosis, seen in over 50% of tumors, and the common occurrence of mitotic activity in order to avoid a mistaken diagnosis of osteosarcoma, particularly on limited biopsy specimens.
36 Fibrous Hamartoma of Infancy- A Clinicopathologic Study of 122 Cases, Including 2 with Malignant Progression

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Background: Fibrous hamartoma of infancy (FHI) is a rare soft tissue lesion of infants and young children with characteristic trichophytic morphology. FHI typically occurs in the axilla and rarely other locations. Although FHI is currently characterized as a “hamartoma”, its morphological features and clinical behavior suggest it may be a neoplasm. Owing to its rarity, the natural history of FHI and its potential for aggressive behavior is not fully defined.

Design: Available slides from 138 cases diagnosed as “FHI” were retrieved from our archives. Sixteen cases were excluded, leaving a final study population of 122 cases. Follow-up was obtained. Selected cases were tested for PDGFB and ETv6 genes rearrangements.

Results: Cases occurred in 92 M and 30 F (mean age: 18 months; range: birth to 14 years) and involved the axilla (n=23), upper arm (n=20), back (n=20), chest (n=10), thigh (n=11), neck (n=10), scrotal/inguinal region (n=9), buttock (n=4), abdomen (n=4), breast (n=3), cheek (n=2), finger (n=2), forearm (n=2), clavicular soft tissue (n=1) and sacral soft tissue (n=1). Four were congenital. The tumors presented as subcutaneous masses and ranged from 0.5-17 cm (mean 3 cm). All displayed trichophytic morphology, although the relative percentages of fat, fibroblastic foci and primitive mesenchyme varied widely. Hyalinized zones with cracking artifact, mimicking giant cell fibroblastoma (GCFB), were present in a subset of cases; however PDGFB FISH was negative in 3 tested cases. In addition to classical FHI, 2 cases contained large sarcomatous foci with high cellularity, high nuclear grade and brisk mitotic activity. One occurred in a 10-month old F as a new mass in a congenital FHI, and the other as a leg mass in a 6-year-old M. ETv6 rearrangement was negative in the tumor from the 10-month-old F. Follow-up (26 patients; range: 1 month-208 months, median: 20 months) showed only 2 local recurrences and no metastases. Extensive local disease in the 10-month-old F with sarcomatous FHI necessitated forequarter amputation.

Conclusions: The majority of FHI show typical trichophytic morphology. A small subset with less obvious trichophytic morphology may be mistaken for various idiopathic fibroblastic and myxoid tumors of childhood. Most notably, sclerotic variants of FHI closely resemble GCFB and may require molecular analysis for PDGFB rearrangement. The identification of sarcoma in the presence of sarcoma within FHI supports the view that these are complex mesenchymal tumors rather than “hamartomas.”

37 Malignant Tenosynovial Giant Cell Tumor- A Clinicopathologic and Immunohistochemical Study of 7 Cases

Alyaa Al-Ibraheemi, Andre Oliveira, Andre L Folpe. Mayo Clinic, Rochester, MN.

Background: Tenosynovial giant cell tumors (TGCT) are relatively common tumors of tendons/joints that grow in localized and diffuse patterns and are characterized by a distinctive admixture of cell types, including synoviocyte-like cells, macrophages, large hemosiderin-laden osteocartilaginous cells and osteoclasts. By immunohistochemistry (IHC), TGCT contain clusterin and desmin-positive cells (likely synoviocytes) and cells expressing histiocytic markers (e.g., CD68, CD163, CD11c). Genetically, TGCT often contain CSF1 rearrangements, typically partnered with COL6A3. Malignant tenosynovial giant cell tumors (MTGCT), consisting of benign TGCT coexisting with sarcoma (primary MTGCT), or representing sarcomatous recurrences of previously diagnosed benign TGCT (secondary MTGCT), are very rare and poorly characterized.

We studied a series of MTGCT in order to better understand their natural history and behavior.

Results: We detected fusion transcript for 9 synovial sarcomas (9/9), 9 alveolar soft tissue sarcomas (9/9), 9 osteosarcomas (9/9), 9 liposarcomas (9/9), 9 desmoplastic small round cell tumors (2/2), 1 dermatofibrosarcoma protuberans (1/1), 1 clear cell sarcoma (1/1), 2 angiomatoid fibrous histiocytomas (2/2), 1 infantile fibrosarcoma (1/1) and 1 solitary fibrous tumor (1/1). For 7 tumors, sequence quality was only reached using cryopreserved tissue. We did not detect transcript for 2 primary bone Ewing sarcomas and 1 dermatofibrosarcoma protuberans. Unfortunately, for these latter tumors we did not have cryopreserved tissue. One myxoid liposarcoma transcript was not detected.

Conclusions: We have developed a multiplexed assay which can reveal a large number of gene fusions in sarcomas with good sensitivity and excellent specificity. These promising results provide an opening for this new rapid simple low-cost multiplexed targeted sequencing assay as an alternative method to FISH and RT-PCR for routine diagnosis.

38 A New Simple Low-Cost Multiplexed Targeted Sequencing Assay to Detect Recurrent Fusion Genes in Sarcomas

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Background: Sarcomas represent a heterogeneous group of tumors comprising more than 50 histological different types. Ten to fifteen percent of sarcomas are characterized by specific chromosomal translocations which are routinely used as molecular markers for diagnosis, providing crucial information for prognosis and therapeutic decision.

Today, translocations are detected by FISH or RT-PCR. However, these methods can only detect a limited number of transcripts simultaneously. Since the development of high throughput sequencing, the number of specific translocations continues to grow and it seems that we have reached the limits of this molecular “shot-gun” approach.

Design: We have developed a simple low-cost (less than 6 dollars per patient) assay based on multiplex ligation-dependent RT-PCR for simultaneous screening of more than 50 rearrangements present in sarcomas. To validate this assay, we selected 42 formalin fixed and paraffin embedded (FFPE) sarcomas with known molecular alteration. In the case of non-contributive results, we repeated the analyses with snap-frozen tissue. Results: We detected fusion transcript for 9 synovial sarcomas (9/9), 9 alveolar soft tissue sarcomas (9/9), 4 Ewing sarcomas (4/6), 2 Ewing like sarcomas with BCOR-CCNB3 fusion transcript (2/2), 6 myxoid liposarcomas (6/7), 2 desmoplastic small round cell tumors (2/2), 1 dermatofibrosarcoma protuberans (1/2), 1 clear cell sarcoma (1/1), 2 angiomatoid fibrous histiocytomas (2/2), 1 infantile fibrosarcoma (1/1) and 1 solitary fibrous tumor (1/1). For 7 tumors, sequence quality was only reached using cryopreserved tissue. We did not detect transcript for 2 primary bone Ewing sarcomas and 1 dermatofibrosarcoma protuberans. Unfortunately, for these latter tumors we did not have cryopreserved tissue. One myxoid liposarcoma transcript was not detected.

Conclusions: We have developed a multiplexed assay which can reveal a large number of gene fusions in sarcomas with good sensitivity and excellent specificity. These promising results provide an opening for this new rapid simple low-cost multiplexed targeted sequencing assay as an alternative method to FISH and RT-PCR for routine diagnosis.

39 TERT Promoter Mutations and Prognosis in Solitary Fibrous Tumor

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Background: Solitary fibrous tumor (SFT) is a mesenchymal neoplasm exhibiting a broad spectrum of biological behaviors. Clinicopathologic parameters are currently used in risk prediction models for SFT, but the molecular determinants of malignancy in SFTs are unknown. We hypothesized that the activation of telomere maintenance pathways confers a perpetual malignant phenotype in these tumors. Therefore, we investigated TERT promoter mutations as a potential underlying mechanism for TERT expression in SFT.

Design: TERT promoter mutational analysis was performed by PCR and Sanger sequencing in samples from 80 patients with SFT of pleural/pulmonary (n=31) or soft tissue (n=49) origin. Telomerase expression was assessed by using reverse transcription quantitative real-time PCR (RTqPCR) in pleural SFTs from 26 patients for whom adequate tumor tissue was available. TERT promoter mutation and telomerase expression status were correlated with clinicopathologic risk groups and outcomes.

Results: Patients were stratified into clinicopathologically high-risk (n=19), moderate-risk (n=24), and low-risk (n=37) subgroups by using the risk stratification model proposed by Demicco et al. Hotspot TERT promoter mutations were identified in 24 (30%) patients with SFT. -13 high-risk, 10 moderate-risk, and 1 low-risk patients. RTqPCR revealed that the presence of TERT promoter mutations correlated with high TERT expression levels (Figure 1).

Outcome data were available so far for 39 patients. In 11 of 15 (73%) patients with a mutant TERT promoter, the SFT behaved aggressively (recurrent disease/death), whereas only 3 of 24 (13%) patients with the wild-type TERT promoter SFT had poor outcomes.
Conclusions: Our data suggest that TERT promoter mutations are the molecular mechanism underlying TERT expression in a majority of malignant SFTs. Adding TERT promoter mutations as a molecular marker to the existing multivariable risk prediction model is expected to improve risk prediction in patients with SFT. The molecular events governing the maintenance of telomeres in the subset of malignant SFTs having the wild-type TERT promoter remain to be determined.

40 Analysis of GNAS Mutations in a Series of Intramuscular Myxomas with Identification of a Novel R201L Mutation
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Background: GNAS mutations have been implicated in the development of tumors associated with McCune Albright Syndrome (MAS, endocrine tumors and fibrous dysplasia [FD]) and in Mazabraud’s syndrome (FD and intramuscular myxomas [IM]). Further, GNAS mutations have been identified in non-syndromic cases of IM (57%) and FD (72%, range 23-100%). Codon 201 exon 8 of GNAS is the most frequently involved with R201H (53% in IM and 66% in FD) and R201C (44% in IM and 31% in FD) being the most common. Other mutations involving codon 201 have been reported in FD and in patients with MAS, but not in IM.

Design: Twenty-two cases of IM were confirmed by H&E and radiographic imaging. Three cases were excluded due to insufficient material. Four cases of juxta-articular myxoma (JA) were used as negative controls. PCR and direct sequencing were used to analyze codon 201 exon 8 GNAS mutation status using genomic DNA isolated from FFPE samples.

Results: GNAS mutations were identified in 10/19 (53%) cases of IM, with R201C (arginine to cysteine) in 8 (80%), R201H (arginine to histidine) in 1 (10%), and a novel R201L (arginine to leucine) in 1 (10%) of the mutated cases. No mutations were detected in the 4 cases of JA.

WT 201R (CGT, Arginine)
F: CGCTGTCG (5’-3’)
R: GGAACAGG (3’-5’)

Mutation 201L (CTT, Leucine)
F: CGCTGTCG (5’-3’)
R: GGAACAGG (3’-5’)

Conclusions: We report the first case of a R201L (arginine to leucine) GNAS mutation identified in IM. Similar to other studies, we identified a significant percentage of cases of IM harboring mutations in GNAS, although we report a higher frequency of R201C mutations. While a R201L mutation has been reported in an individual with MAS, it has yet to be reported in IM or FD. Our study, in addition to others, did not investigate other GNAS mutation hotspots reported in patients with MAS and FD such as codon 227. This raises the possibility that GNAS mutations are more prevalent in IM than currently thought. Further investigation is needed to evaluate IM for other less common GNAS mutations.

41 A Novel Subgroup of Deep Myxoma with Specific Genetic and Histologic Findings and No Evidence of GNAS Mutations: Analysis of Three Cases
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Background: Deep myxomas (intramuscular [IM] and juxta-articular [JA]) are benign tumors that most commonly occur in the deep tissues of the extremities in adults. The tumors may reach a large size but do not undergo malignant transformation. Recurrence risk is minimal for IM but approximately 35% for JA. The two entities may be indistinguishable on histologic review. Point mutations of the GNAS gene are commonly found IM (~40-50%) but are absent in JA. Few cytogenetic findings in deep myxomas have been reported. We recently identified a subset of deep myxomas with increased desmin expression and aberrations of 17q21-22. We performed additional molecular analysis of these cases to better understand them.

Design: Three cases of deep myxomas (2 IM and 1 JA) were identified that showed increased desmin IHC expression and contained alterations of 17q21-22 (Table 1). FISH analysis was performed on these tumors targeting COL1A1 on 17q22 using BAC clone RP11-267M22 to evaluate for a translocation in this region. GNAS mutation analysis of codon 201 exon 8 was performed using PCR and direct sequencing of genomic DNA isolated from FFPE.

Results: FISH analysis of COL1A1 failed to identify a translocation. Evaluation of codon 201 of GNAS showed the wild type sequence CTG coding for arginine in all three cases (Figure 1).

WT 201R (CGT, Arginine)
F: CGCTGTCG (5’-3’)
R: GGAACAGG (3’-5’)

Conclusion: These findings provide further evidence of a genetically distinct myxoid soft tissue tumor with increased desmin expression and aberrations of 17q21-22. FISH analysis of the COL1A1 gene associated with dermatofibrosarcoma protuberans was negative, excluding a myxoid variant of this entity. The absence of GNAS mutations in these cases does not necessarily exclude these tumors being variants of IM myxoma. However, the absence of GNAS mutations, the presence of desmin positive fibroblasts and the fact that this unique group comprises cases diagnosed as IM and JA myxoma based on clinical and imaging suggests the possibility of another entity with histologic features overlapping those of IM but with similar indolent behavior.

42 Pediatric Non-Vestibular Schwannomas: A Clinicopathologic Study of 22 Patients
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Background: While the clinicopathologic features of pediatric vestibular schwannomas, often in the context of neurofibromatosis type 2 (NF2), have been well-studied, there is less data regarding the characteristics of pediatric non-vestibular schwannomas (NVS). Additionally, the rate of SMARCB1/INI1 loss in this population has not been systematically evaluated.

Design: Our institutional archives were searched for cases of NVS arising in patients 18 years or younger. Pathologic characteristics assessed in each case included size of tumor, percent Antoni A and Antoni B tissue, degenerative atypia, calcifications, plexiform architecture, microcystic/reticular growth, mitotic activity, small cell features, and necrosis. Immunohistochemistry for SMARCB1/INI1 protein was performed on a representative block from each case. Clinical information collected included age, sex, location of tumor, and personal/family history of schwannomas or genetic syndromes.

Results: Out of 1960 cases of schwannoma reviewed, 23 pediatric NVS from 9 males and 13 females (age range 5 months to 18 years) were identified; sites included paraspinal (n=10), head and neck (n=6), extremities (n=4), trunk (n=1), mediastinum (n=1) and retroperitoneum (n=1). 21 cases were Antoni A predominant (70 to 100%) with 8 cases comprised solely of Antoni A tissue. 5 cases had plexiform architecture. The mitotic rate of the tumors ranged from 0 to 10/10 high power fields (HPFs), and 3 tumors had mitotic rates of 2-4 mitoses/10 HPFs. No NVS showed diffuse degenerative atypia, calcifications, microcystic/reticular architecture, small cell features, or necrosis. Two patients had NF2, and both of those patients had multiple NVS. 3 additional patients had multiple NVS. Followup was available in 21 patients (range 1 week to 194 months), and 4 patients (1 NF2, 3 non-NF2) experienced a recurrence or developed additional NVS. All tumors (23/23) showed retained nuclear expression of SMARCB1/INI1.

Conclusions: Pediatric NVS are uncommon and seem to have a relatively homogeneous appearance, with little histomorphologic variability, and a predominance of Antoni A areas. Pathologists should be aware that schwannomas in this age group may be cellular with mitotic rates > 4/10 HPFs to avoid misclassification as spindle cell sarcoma. Loss or mosaicism of SMARCB1/INI1 appears uncommon in this subset of tumors, but a larger series is needed to confirm these findings.
43 Extraosseous, Extradural Chordomas of the Spine: A Unique Chordoma Subtype
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Background: Chordomas are malignant tumors derived from the embryologic notochord. The vast majority arise within the bones of the skull base and spine. While chordomas arising in extraspinal bone and soft tissues have been described, there are only rare case reports of extradural chordomas in the radiology and neurosurgery literature, and none in the pathology literature. We report our experience, over the past two decades, with six cases of extraosseous, extradural chordomas presenting with a unique clinicoradiologic profile.

Design: Six cases of extraosseous, extradural chordoma were retrieved from our institutional archives. Clinical history, radiologic studies and histologic slides were evaluated. Clinical follow-up was obtained from the medical record. On initial imaging, a lesion closely mimicking a benign neurogenic tumor, composed of a unihematous enhancing extradural mass (mean 2 cm), expanding and extending through neural foramina within the cervical (N=6) or lumbar (N=2) spine. No case showed intraspinous extension. All tumors were surgically excised (2 with a pre-operative diagnosis of chordoma), with variable surgical margins. One patient received adjuvant external beam radiotherapy and two patients received proton beam therapy. Histologically, all 6 tumors showed typical features of conventional chordoma including epithelioid, vacuolated cells with variable amounts of eosinophilic cytoplasm, forming cords and nests, embedded in a myxoid matrix. In tested cases, the neoplastic cells uniformly expressed wide-spectrum cytokeratins (N=6) and brachyury (N=4). With mean follow-up of 6.4y (range 1-20y), one patient had local residual/recurrent disease within 1 year. The other 5 patients are disease-free.

Results: We report the first series of extraosseous, extradural chordomas. This rare subset of chordoma has a distinctive and misleading clinicoradiologic profile, closely resembling a benign neurogenic tumor. It is important for pathologists to be aware of this entity to avoid a mistaken diagnosis of soft tissue tumors more commonly seen in this setting, and to guide the appropriate oncologic management indicated for chordomas.

44 Identification of Novel Gene Fusion FUS-CREM in Clear Cell Sarcoma of Soft Tissue by Anchored Multiplex Polymerase Chain Reaction
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Background: Gene fusions EWSR1-ATF1 and EWSR1-CREB1 have been identified in CCS, identification of which is often crucial in distinguishing CCS from malignant melanoma. Anchored multiplex polymerase chain reaction (AMP) is a next-generation sequencing-based technique in which gene fusions can be identified in a PCR-based assay for which there are sarcoma-targeted panels available (Archer FusionPlex Sarcoma Panel). This assay has discovery potential, as it utilizes primers targeting only one fusion partner, while the other partner is identified through next-generation sequencing agnostic of the identity and breakpoint of the binding partner. We describe a novel gene fusion FUS-CREM in a CCS which was identified by AMP.

Design: The patient was a 55 year old male with a thigh mass. Morphology and immunohistochemistry supported a differential diagnosis of CCS versus malignant melanoma, and EWSR1 FISH was performed during initial diagnostic work-up. AMP was performed on this case as part of a validation study of the technology in sarcomas diagnostics. We also performed FISH utilizing a FUS breakapart probe, and RT-PCR with primers for FUS and CREM. A series of 8 additional EWSR1-intact melanocytic tumors in which CCS was a differential diagnosis were further assessed by FISH for the FUS-CREM fusion.

Results: Initial EWSR1 FISH showed a complex polyplody result with no EWSR1 gene rearrangement identified. AMP demonstrated a novel FUS-CREM fusion, with the fusion junction between FUS exon 8 and CREM exon 7. FISH confirmed a rearrangement of the FUS locus, and RT-PCR demonstrated an amplicon across the exon 8-exon 7 breakpoint. This finding is biologically justified by the homology between CREM and CREB1, both representing cyclic AMP-responsive element modulators. Amongst the 8 additional cases evaluated, no further cases showed the FUS-CREM gene fusions.

Conclusions: FUS can replace EWS in CCS. We identify a novel gene fusion FUS-CREM in CCS using the AMP technique, adding to the list of known gene fusions characterizing CCS. We also show that a targeted next-generation sequencing-based assay such as AMP has the capability of discovering novel gene fusions in which only one fusion partner is covered in the primer pool. Such assays are of increasing diagnostic utility with the ongoing discovery of novel gene fusions and fusion variants.

45 Histopathology of So-Called Synovial Cysts of the Lumbar Spine
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Background: "Synovial cysts" of the lumbar spine are a feature of degenerative arthropathy of facet joints of the lumbar spine. The histogenesis of these specimens is uncertain as many lack true synovial lining, as would be expected for cysts derived from durahttional joints. The purpose of this study was to further characterize the histologic findings of specimens submitted as lumbar "synovial cysts".

Design: The laboratory information system was searched for lumbar “synovial cyst” diagnoses reviewed in 2012-2015. The histologic slides were reviewed with special attention to the lining of the cysts and the presence/absence of the following histologic features was evaluated: evidence of cyst histologically and type of lining; presence of limigmentum flavum (LF); type of cyst contents (fibroinid, myxoid, bloody); presence of calcification and type (dystrophic, calcified, or calcification of calcified). The presence/absence of these features was used to establish a diagnostic algorithm for these lesions.

Results: Seventy-five cases of lumbar “synovial cysts” were reviewed: 54% females, average age 64 (range 50-89) and 46% males, average age 69 (range 51-83). Thirty-one of 75 lumbar cysts (41%) were lined by synovium, at least focally, consistent with synovial cysts. Thirty-six (48%) lumbar cysts lacked synovial lining. Of these, 28/36 cases contained fragments of LF with pseudocyst formation (cytic degenerative changes) and 8/36 showed fibrous-walled pseudocysts without LF. In the remaining 8 patients no cyst was identified histologically. There were 25 cases that had fibroinid cyst contents (7 synovial cysts, 11 degenerated LF, 4 pseudocysts without LF, 3 cases without cyst walls). 5 cases with myxoid contents (3 degenerated LF, 2 cases without cyst walls) and 17 cases of mixed fibroinid/myxoid cyst contents (5 synovial cysts, 10 degenerated LF, 2 cases without cyst walls). Twenty cases (1 synovial cyst, 15 degenerated limigmentum flavum, 4 cases without cyst walls) contained peculiar finely granular basophilic material (staining with von Kossa, when performed) and surrounding foreign-body giant cell reaction, resembling calcifications seen in tumoral calcinosis. These lesions were designated "synovial cysts". They represent a histologic finding and comprise both synovial-lined cysts and non-synovial pseudocysts, often cystically degenerated limigmentum flavum, that may contain finely granular tumoral calcinosis-like calcifications.

46 Diagnostic Utility of IDH1/2 Mutation To Distinguish Dedifferentiated Chondrosarcoma from Undifferentiated Pleomorphic Sarcoma
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Background: Histologically, it is impossible to distinguish dedifferentiated chondrosarcoma (CHS) from undifferentiated pleomorphic sarcoma (UPS) of the soft tissue. In previous studies, we identified an IDH1 mutation signature which could be used as a clinically diagnostic marker for distinguishing these two lesions.

Design: Cases of dedifferentiated CHS and UPS were collected from Indiana University, Mayo Clinic and University of Alabama at Birmingham. H. E. slides were re-examined with diagnoses were confirmed. DNA was extracted from formalin fixed paraffin embedded tissues. IDH1/2 mutation analysis was performed using the Qiagen IDH1/2 RQG PCR Kit, which detected multiple mutations in codon 132 of the IDH1 gene and codon 172 of the IDH2 gene, respectively.

Results: Among 23 cases of dedifferentiated chondrosarcoma,18 each harbored one somatic mutation in either IDH1 or IDH2. Specifically, they were 5 R132C, and 4 R132 mutations in IDH1 gene and 9 R172 mutations in IDH2 gene. Interestingly, two cases each contained mutations in both IDH1 and IDH2. They are IDH1 R132 and IDH2 R172 in one case and IDH1 R132H and IDH2 R172 in the other case. Among 24 cases of UPS of the soft tissue, no mutations were detected in the IDH1 or IDH 2.

Conclusions: Overall, 20/23 (87%) dedifferentiated CHS cases contain at least one mutation in either IDH1 or IDH2. Our study shows higher mutation rate compared to one previous study 13/23 (56.5%) in the dedifferentiated CHS. Identification of IDH1 or IDH2 mutation strongly supports the diagnosis of dedifferentiated CHS rather than UPS.

47 Ewing Sarcoma (ES) with ERG Gene Rearrangements: A Molecular Study Focusing on the Prevalence of FUS-ERG and Common Pitfalls in Detecting EWSR1-ERG Fusion via FISH
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Background: The genetics of ES are characterized by a canonical fusion involving EWSR1 gene and a member of ETS family of transcription factors, such as FLII and ERG. In fact ETS gene rearrangements represent the second most common molecular alteration, with EWSR1-ERG being identified in 5-10% of cases and only a handful of reports documenting a FUS-ERG fusion.

Design: In this study, we focus on ES with ERG gene abnormalities, specifically investigating the prevalence and clinicopathologic features of FUS-ERG fusions in a large cohort of small blue round cell tumors (SBRTs) and comparing them to the FUS-positive ES from the literature.

Results: Among the 80 SBRTCs tested, 6 (7.5%) cases harbored FUS gene rearrangements, 5 showing fusions with CAP and 1 with TCF11. Among 5 cases, if nuclear FISH with a fusion EWSR1-ERG probe was used, 4 cases were negative for the fusion signal. We further identified a number of ES with ERG gene rearrangements by FISH lacking both EWSR1 and ERG abnormalities. In one case RNA sequencing performed showed an EWSR1-ERG transcript despite lack of EWSR1 rearrangements by FISH. Additional 3-color FISH fusion assay demonstrated the fusion of EWSR1 and ERG signals in the 4 cases negative for break-apart EWSR1. These masked fusions occur as EWSR1 and ERG have opposite orientations, requiring a complex rearrangement and/or translocation to produce a genetic readthrough fusion. These ES might allow the development of molecular-targeted therapy for dedifferentiated CHS.
EWSR1-ERG fusion, where FISH assay did not detect EWSR1 rearrangements in half of the cases due to the complex pattern of G21(22). In cases with classical morphologic appearance and/or strong CD99 and ERG immunoreactivity, additional molecular testing should be applied, such as EGR FISH or RT-PCR/NGS, for a definitive diagnosis. Although our study group is limited there were no clinical, morphologic and immunoprofile differences noted among the various subsets of EGR-rearranged SBCRTs.

48 MDM2 and CDK4 Immunohistochemistry is Inadequate in the Diagnosis of Problematic Low-Grade Lipomatous Tumors: A Follow-Up Study

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Background: Immunohistochemistry (IHC) has been promulgated as a surrogate marker for fluorescence in situ hybridization (FISH) testing in atypical lipomatous tumor/well differentiated liposarcoma (ALT/WDL). However, this has not been validated in a large series of cases with either bland or borderline histologic features that specifically could not have been diagnosed based on H&E staining alone. We have evaluated the utility of IHC for MDM2 and CDK4 in borderline cases with known MDM2 FISH results.

Design: Immunohistochemical staining for MDM2 and CDK4 was performed in 210 low-grade lipomatous tumors with known FISH results. 27 cases were removed secondary to scant tissue on stained slides or poor staining quality (uninterpretable), leaving 183 cases (56 ALT/WDLs and 127 lipoma/lipoma variants). Two pathologists blinded to the FISH results scored the slides, with discrepant cases discussed and consensus reached. Each stain was considered positive if >10% of tumor nuclei displayed 1+ staining, or if <1% of tumor nuclei displayed 2-3+ intensity.

Results: We observed expected non-specific staining of histiocytes and endothelial cells. A number of cases also showed false positive staining of tumor nuclei (n=9, 2 pleomorphic lipomas, 7 lipomas), some of which was strong and diffusely (up to 75% of tumor nuclei). 28 cases exhibited very rare faint staining (1-5%, 1+). These were considered negative at the time of interpretation, and a retrospective review indicated these cases were typically negative by FISH (24 of 28 cases, 85%). The test characteristics can be seen in table 1.

| MDM2 IHC | CDK4 IHC | Used in Combination |
|----------|----------|---------------------|
| Positive  | Positive  | Positive/Negative   |
| Positive  | Positive  | Positive/Negative   |
| Positive  | Negative  | Positive/Negative   |
| Negative  | Positive  | Positive/Negative   |
| Negative  | Positive  | Positive/Negative   |

Conclusions: IHC is insufficiently sensitive for routine diagnosis ofdiagnostically challenging lipomatous tumors. Higher sensitivity in previous studies likely results from greater cellularularity than was present in our paucicellular lipoma-like cases, but it is in these latter cases that ancillary testing is most needed. In histologically bland and borderline adipocytic tumors, FISH for MDM2 gene amplification should be the first line ancillary test.

49 HER3 (erbB3) as a Potential Therapeutic Target in Peripheral Nerve Sheath Tumors (PNST): In Vitro and In Vivo Models

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Background: Malignant peripheral nerve sheath tumors (MPNST), comprising 5-10% of soft tissue sarcomas, are associated with neurofibromatosis type 1 (NF1) and sporadic lesions. HER3 expression was also evaluated by western blot in 5 mesenchymal cell lines with known HER3 expression. Monophasic histologies had significantly higher expression than biphasic (59% vs. 38% of sarcoma nuclei).

Conclusions: HER3 overexpression is frequently found in MPNST. Genetic and pharmacologic approaches to blockade HER3 in MPNST are able to prevent tumor growth only in the subgroup of tumors overexpressing HER3. This supports the rationale of the use of HER3 inhibitors/monoclonal antibodies as new therapeutic approaches for the treatment of MPNST.

50 KDM2B—Setting the Stage for Abrupt Silencing in Synovial Sarcoma

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Background: Recent studies have highlighted a critical role for epigenetic dysregulation in synovial sarcoma, which may be potentially targetable with drugs under active development. Synovial sarcoma occurs in part as a result of tumor suppressor gene silencing due to genome-wide hypomethylation. This silencing is brought about by the disease’s pathognomonic fusion oncogene, SS18-SSX, which links DNA-binding transcription factor ATF2 to gene-silencing complex PRC2; however, the epigenetic background of synovial sarcoma likely involves other cofactors.

Our group, using a high throughput RNAseq screen for epigenetic cofactors, identified KDM2B as a gene as critical to synovial sarcoma growth as SS18-SSX. KDM2B is a histone demethylase specific to histone-3-lysine-36 (H3K36). Recent work suggests that H3K36 methylation prevents PRC2-mediated H3K27 methylation, explaining why knockdown of KDM2B might be necessary for SS18-SSX-driven tumor suppressor silencing. Indeed, we recently confirmed high KDM2B mRNA expression in synovial sarcoma cell lines, and found that knockdown of SS18-SSX induces KDM2B protein depletion; however, the mechanism of KDM2B tumorigenicity in synovial sarcoma has not yet been confirmed in patients.

Design: KDM2B immunohistochemistry was performed on paraffin-embedded tissue microarrays containing samples from 57 human synovial sarcomas. For comparison, staining was also done on arrays containing over 200 other soft tissue neoplasms: translocation-associated sarcomas, other sarcomas, and benign mesenchymal tumors. Results were linked to treatment and outcome data.

Results: KDM2B gave easily interpretable, strong nuclear staining in all synovial sarcoma cases. A mean of 54% of synovial sarcoma cells were positive (95%CI 51-57), significantly higher (p<0.0002) than the combined mean across other neoplasms studied, among which benign nerve sheath tumors and melanomas also showed relatively high expression. In synovial sarcoma, high expressors of KDM2B had improved overall survival at 15 years, with a hazard ratio of 0.11 at the optimized cutoff (92% expression). Monosomatic histologies had significantly higher expression than diphasic (59% vs. 38% of sarcoma nuclei).

Conclusions: This data demonstrates that KDM2B, a potential therapeutic target for new epigenetic modifying drugs, is commonly and highly expressed in patient synovial sarcoma specimens. This work adds to an emerging body of evidence for a role of KDM2B and epigenetic dysregulation in the oncogenesis of synovial sarcoma.

51 Identification of Recurrent Copy Number Alterations Specific to the De-Differentiated Component of Liposarcoma, Using Capture-Based Next Generation Sequencing (NGS)

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Background: Well-differentiated liposarcoma (WL) is the most common soft tissue sarcoma in adults. WL provides a unique model of sarcoma progression, in that “dedifferentiation” of WL to a non-lipogenic, or de-differentiated, liposarcoma (DL) is well-documented. WL and DL demonstrate amplification of chromosomal bands 12q13-15 but the mechanism of progression from WL to DL is unclear. Increased activity of AP1 signaling has been proposed as a mechanism in some cases. In the present study, we compared single nucleotide variants (SNVs), small insertions or deletions (Indels), and copy number alterations (CNAs) between matched lipogenic and non-lipogenic components of DL.

Design: Ten WL with matched normal, lipogenic and non-lipogenic components were selected. DNA was extracted from formalin-fixed paraffin embedded tissue. Capture-based next generation sequencing (NGS) was performed targeting the coding regions of over 500 cancer genes as well as selecting introns, covering a total of 2.8 Megabases (MB).

Results: Average sequencing depth was 635 unique reads per interval. No recurrent SNVs or small Indels were identified that could account for progression, including in AP1 signaling pathway genes. There was also no difference in mutational burden between matched lipogenic and non-lipogenic pairs (2.9 mutations/MB and 3.1 mutations/MB, respectively). Recurrent CNAs were identified, including the expected amplification of 12q15 (MDM2) and 12q13.3 (CDK4) in both components of all 10 cases. Amplification of 8q23 (MAPK3) was also seen in matched lipogenic and non-lipogenic components in 4 samples.

Conclusions: DNA copy number alterations, especially amplifications of 1p13 (JUN), 11p12 (RAF2), and 8q21, and deletions of 11q, 10q (PTEN), distal 11q and 3q21-22.

| Amplifications | Deletions |
|----------------|----------|
| JUN            | PTEN     |
| RAF2           | PTEN     |
| 3q21-22        |          |
| Lipogenic      | 0        |
| Non-lipogenic  | 5        |
|                | 4        |
|                | 5        |
|                | 3        |
|                | 3        |
Mutations in Histone H3.3 Variants in Giant Cell Tumor of Bone
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Background: Giant cell tumor of bone (GCT) is a benign, locally aggressive, neoplasm associated with a high rate of local recurrence. While in young adults, encompass a broad anatomic distribution, and may be associated with significant morbidity. The disease-defining mutation in GCT frequently involves histone 3.3 (H3F3A), with most cases associated with G34W, and rare cases with G34V, G34R, and G34M mutations. There remains a minority of cases with an hitherto unreported underlying driver alteration; as a result, we sought to analyze our own patient population for histone H3F3A mutations.

Methods: Cases were re-reviewed to confirm the diagnoses. Using digital droplet PCR (ddPCR) we analyzed 23 consecutive GCTs for H3F3A mutations. Briefly, formalin fixed-paraffin embedded tissue was cut into 10 micron sections, treated with deparaffinization solution and DNA extracted. ddPCR was performed according to standard methods with primers and probes specific for G34W and mutant allele frequency was calculated. Samples that did not carry G34W mutation in H3F3A were analyzed by Whole Exome Sequencing using Illumina Nextera Rapid Exome.

Results: A driver mutation in histone H3.3 was identified in 100% of cases, with the vast majority being in H3F3A.G34W (82.6%), and minor incidence for G34V (8.7%) and G34R (4.3%). We also identified an additional alternate mechanism affecting H3.3 in the sample that was wildtype for G34 H3.3.

Conclusions: Elucidation of the genetic alterations in GCT offers the potential for both refined diagnostic capabilities for pathologists, as well as potential therapeutic targets. We confirm the high prevalence of the G34W mutation in GCT. Our results also highlight the presence of G34L and G34V mutations in a minority of cases. We also include alterations in H3.3 drive virtual tumor panel testing to describe the functional impact of these G34X/H3.3 alterations in the genetics of GCT.

55 A Subset of Epithelial Sarcomas with Intact INI1 (SMARCBI) is Deficient for SMARC4A and SMARC2
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Background: Approximately 5% of epithelial sarcomas, both conventional and proximal types, show intact INI1 (SMARCBI) expression by immunohistochemistry (IHC). Little is known about the molecular genetics of this small subset of epithelial sarcomas. SMARC4A is the ATPase subunit of the SWI/SNF chromatin-remodeling complex. Recently, SMARC4A mutations were identified in ovariian small cell carcinoma with pericentric-type, some undifferentiated thoriocarcinomas with epithelioid or rhabdoid features, and a small subset of malignant rhadoblastomas (with intact INI1 expression). Corresponding loss of SMARC4 protein expression and concomitant loss of SMARC2 (another member of the SWI/SNF complex) are also seen in these tumor types. The goal of this study was to determine if SMARC4A/SARC2A deficiency was present in epithelial sarcomas with intact INI1 expression.

Design: 15 cases of epithelial sarcoma with intact INI1 expression, but otherwise typical morphologic and immunohistochemical features, were identified (9 conventional type, 6 proximal type). IHC for SMARC4A and SMARC2A was performed on whole tissue sections; expression was considered deficient when tumor cells lacked nuclear staining in the presence of nuclear reactivity in non-neoplastic cells, which served as internal controls. Clinical and histologic features were also reviewed.

Results: 2 cases showed loss of both SMARC4A and SMARC2A expression. Both tumors arose in males (40 and 61 years of age), one in a paraspaseolmediastinal location and the other in the retroperitoneum; the latter patient also had a larger pleural-based mass and a smoking history. Tumors were composed of sheets of large, monomorphic epithelial cells with abundant amphiphilic or palely eosinophilic cytoplasm and round nuclei with prominent nucleoli, and showed extensive tumor necrosis. Focal rhabdoid features were seen in one case. Both tumors expressed keratins, EMA and CD34. Both patients had metastases at the time of presentation.

Conclusions: A subset of proximal-type epithelial sarcomas with intact INI1 expression is deficient for SMARC4A and SMARC2A. These tumors show significant overlap with the recently described ‘SMARC4A-deficient thoriocarcinoma’ distinguished from proximal-type epithelial sarcoma only by intact INI1 expression and loss of SMARC2A/SARC2A, the latter due to somatic SMARC4A mutations.

56 Histone 3.3 Mutations in Giant Cell Tumor and Giant Cell-Rich Sarcoma of Bone
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Background: Mutually exclusive histone 3.3 gene mutations have been recognized in chondroblastoma and giant cell tumor of bone (GCTB), which may be useful for differential diagnostic purposes in morphologically ambiguous cases. While over 90% of typical GCTB presents histone 3.3 variants exclusively in the H3F3A gene, chondroblastoma is mutated mainly in H3F3B.

Design: In this study we examined a series of giant cell rich primary bone tumors, aiming to evaluate the possible diagnostic role of histone 3.3 mutations in the differential of giant rich sarcomas. Fifteen cases of GCTB and 15 giant cell-rich sarcomas (8 osteosarcomas and 7 undifferentiated pleomorphic sarcomas) were selected from our institutional archives. Eight chondroblastomas were used as controls. Direct sequencing for the presence of H3F3A and H3F3B variants in coding region between codons 1 and 42, including the hot spot codons (28, 35 and 37) was performed on DNA extracted from formalin-fixed paraffin-embedded tissue.
Results: Forty-five GCTs (93.3%) presented a mutation in the H3F3A gene (11 G3SW, 1 G3SY, 5 G3SM and 1 G3SE). In the group of sarcomas, we identified two variants. One was a G3SE in a secondary malignant GCTB, which developed after 4 local relapses of a GCT of the sacrum, and the second was a G3SW in a giant cell rich osteosarcoma. No mutation was identified in the H3F3B gene in the group of GCTB and giant cell-rich sarcomas. No mutations in the chondroblastoma tested presented a robust variant.

Conclusions: Our results confirm that H3F3A mutations occur more frequently in GCTB, while they are seldom found in giant cell rich sarcomas of bone arising de novo. Thus, H3F3A mutational testing may be a useful adjunctive to differentiate GCTB from giant cell rich sarcomas.

57 Detection of Sarcoma Oncogenic Fusion Transcripts Using ArcherTM Sequencing Technology

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Background: Despite recent advances, the genetic abnormalities of most primary sarcomas are not known. Gene amplifications, with a subset of cases harboring known oncogene alterations, may provide insight into tumor biology.

Methods: We analyzed a total of 120 cases, 40 liposarcoma, 20 adipocytic sarcoma, 20 angiosarcoma, 20 synovial sarcoma, 20 leiomyosarcoma, 20 rhabdomyosarcoma, 20 Ewing sarcoma, and 20 other sarcomas, which may provide insight into tumor biology.

Results: ArcherTM F3E3 fusion with identical breakpoints was detected in 4 in 14 colorectal cancers, 4 in G3A/3S muscle tumors, 4 in renal cell carcinomas, 4 in breast carcinomas, 4 in prostatic carcinomas, 4 in chordee sarcomas, and 4 in small cell carcinoma. All 4 of these cases were positive for TFE3 by IHC.

Conclusions: The ArcherTM F3E3 fusion is a common oncogenic fusion gene detection methods, but each has its disadvantages. ArcherTM, RNA-based next-generation sequencing assay, allows targeted oncogenic fusion transcript detection without knowledge of the corresponding fusion partners or breakpoints. In this study, we compared the performance of ArcherTM to other methods in various sarcomas with well-characterized fusion genes.

58 Recurrent Novel CIC Gene Abnormalities in Angiosarcoma: A Molecular Analysis of 60 Cases with Concurrent Investigation of PLCG1, KDR, MYC, and FLT4 Gene Alterations

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Background: Angiosarcoma (AS) is a rare sarcoma subtype showing considerable clinicopathologic and genetic heterogeneity. Most commonly, AS occurs as a consequence of chronic inflammation, with a subset of cases harboring KDR, PPPR8 and PLG1 gene amplifications. Despite these recent advances, the genetic abnormalities of most primary AS remain undefined.

Results: Whole transcriptome sequencing was initiated in 2 index cases of primary soft tissue AS with undifferentiated morphology occurring in young adults for novel gene discovery. The candidate abnormalities were validated and then screened by targeted sequencing and FISH in a large cohort of 120 well-characterized AS. Findings were subsequently correlated with the status of KDR, PLG1, MYC and FLRT4 gene abnormalities. The clinicopathologic relevance and prognostic significance of these genetic changes were analyzed by statistical methods.

Results: Concurrent G35 mutations and CIC transcript alterations were identified in both index cases, with a CIC-LEUTX fusion being detected in one. Upon screening, an additional visceral AS in a young adult had a complex CIC rearrangement, while 6 others harbored only CIC mutations. All 3 CIC-rearranged AS lacked vasoformation and had a solid growth pattern, showing no detection rate by FISH. With this NGS-based approach, we were able to discover two novel USP6 fusion partners (RUNX2 and PAFH1B1), which are not amendable to detection by FISH. PAFH1B1 mutation leads to lissencephaly associated with Miller-Dieker syndrome. RUNX2 is a transcription factor involved in neuroendocrine/glial differentiation and a downstream target of EWSR1-FLI1, has been reported as an immunohistochemical (IHC) marker for ES. We assessed the specificity of NCK2-2 for ES compared to other ES-like sarcomas and its utility in other soft tissue tumors with EWSRI-FLI1 translocation.

Conclusions: We evaluated whole-tissue sections from 270 cases: 40 ES (4 with atypical large cell features), 20 CIC-DUX4 sarcomas, 5 BCOR-CXR1 sarcomas, 9 unclassified round cell sarcomas, 10 poorly differentiated synovial sarcomas, 10 lymphoblastic lymphomas, 10 alveolar rhabdomyosarcomas, 10 embryonal rhabdomyosarcomas, 10 Merkel cell carcinomas, 10 small cell carcinomas, 20 melanomas, 5 NUT midline carcinomas, 10 Wilms tumors, 10 neuroblastomas, 10 olfactory neuroblastomas, 12 mesenchymal chondrosarcomas, 10 angiomatoid fibrous histiocytomas (AFMHEL), 10 clear cell sarcomas (CCS), 5 gastrointestinal neuroectodermal tumors (GNET), 5 desmoplastic small round cell tumors (DSRCT), 10 extraleukemic myoid chondrosarcomas (EMCS), 5 angiosarcomas, and 5 mesenchymal chondrosarcomas.

Conclusions: As a sensitive and relatively specific IHC marker for ES, NCK2-2 may be helpful to distinguish ES from most histologic mimics including CIC-DUX4 and BCOR-CXR1 sarcomas. Most other ES-associated soft tissue tumors were negative for NCK2-2, apart from 1 DSRCT, 1 myoepithelioma, and 1 myxopapillary ependymoma.

59 Evaluation of NCK2-2 Expression in Round Cell Sarcomas Including Those with CIC-DUX4 and BCOR-CNXN3 Fusion and Tumors with EWSRI-FLI1 Rearrangement: Relative Sensitivity for Ewing Sarcoma

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Background: Ewing sarcoma (ES), a round cell sarcoma predominantly arising in bone, is a soft tissue of children and adolescents, shows histologic overlap with other round cell tumors. About 90% of ES harbor EWSRI-FLI1 gene rearrangement. NCK2-2, a homeodomain transcription factor involved in neuroendocrine/glial differentiation and a downstream target of EWSRI-FLI1, has been reported as an immunohistochemical (IHC) marker for ES. We assessed the specificity of NCK2-2 for ES compared to other ES-like sarcomas and its utility in other soft tissue tumors with EWSRI-FLI1 translocation.

Design: We evaluated whole-tissue sections from 270 cases: 40 ES (4 with atypical large cell features), 20 CIC-DUX4 sarcomas, 5 BCOR-CXR1 sarcomas, 9 unclassified round cell sarcomas, 10 poorly differentiated synovial sarcomas, 10 lymphoblastic lymphomas, 10 alveolar rhabdomyosarcomas, 10 embryonal rhabdomyosarcomas, 10 Merkel cell carcinomas, 10 small cell carcinomas, 20 melanomas, 5 NUT midline carcinomas, 10 Wilms tumors, 10 neuroblastomas, 10 olfactory neuroblastomas, 12 mesenchymal chondrosarcomas, 10 angiomatoid fibrous histiocytomas (AFMHEL), 10 clear cell sarcomas (CCS), 5 gastrointestinal neuroectodermal tumors (GNET), 5 desmoplastic small round cell tumors (DSRCT), 10 extraleukemic myoid chondrosarcomas (EMCS), 5 angiosarcomas, and 5 mesenchymal chondrosarcomas.

Conclusions: As a sensitive and relatively specific IHC marker for ES, NCK2-2 may be helpful to distinguish ES from most histologic mimics including CIC-DUX4 and BCOR-CXR1 sarcomas. Most other ES-associated soft tissue tumors were negative for NCK2-2, apart from 1 DSRCT, 1 myoepithelioma, and 1 myxopapillary ependymoma.

60 Anchored Multiplexed PCR for Targeted Next Generation Sequencing Reveals Recurrent and Novel Gene Fusions in Aneurysmal Bone Cyst and No Fusion in Giant Cell Tumor of the Bone

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Background: Primary aneurysmal bone cyst (ABC) is now considered a neoplastic process due to recurrent translocations involving the USP6 gene at the short arm of chromosome 17 (1p13). By fluorescence in situ hybridization (FISH), up to 99% of primary ABCs were found to harbor USP6 translocations. USP6 translocation was found in secondary ABC or other histological mimics such as giant cell tumor (GCT) of the bone. The most common translocation in ABC, t(16;17)(q22;p13), fuses EWSR1 next to a highly active CDH11 promoter to upregulate USP6 expression. With this NGS-based method, we were able to discover recurrent and novel gene fusions in a total of 12 cases.

Conclusions: As a sensitive and relatively specific IHC marker for ES, NCK2-2 may be helpful to distinguish ES from most histologic mimics including CIC-DUX4 and BCOR-CXR1 sarcomas. Most other ES-associated soft tissue tumors are also negative for NCK2-2.
Epithelioid schwannoma is rare and may be difficult to recognize. Chromatin remodeling and methylation/demethylation genes were described in the stroma of epithelioid schwannoma. The diagnosis is challenging in the setting of other diagnoses such as angiosarcoma, owing to its distinctive anastomosing, non-lobular pattern of growth, and it is a neoplasm originally described in the kidney, may be confused with well-differentiated meningioma. Some cases show notable cytologic atypia which may indicate a morphologic continuum from meningioma to epithelioid malignant peripheral nerve sheath tumor (MPNST). The male to female ratio was 1.5:1. Follow-up time ranged between 3 and 30 months. Overall, 101 LMS (47%) showed loss of nuclear staining for ATRX. 33% of tumors had loss of ATRX compared to 45% of all advanced disease (p=0.0057). More uterine primaries had loss of ATRX (52%) than soft tissue primaries (21%) (p=0.0001). ATRX was lost more in poorly differentiated tumors (53%) than moderately (39%) and well-differentiated ones (24%) (p=0.0066). The loss of ATRX correlated with earlier tumor recurrence (p=0.06), but not worse survival (p=0.54).

Conclusions: Loss of ATRX can be seen in a significant proportion of leiomyosarcomas with advanced disease, uterine origin and poor differentiation. Furthermore, the loss of ATRX may portend a more aggressive local disease.

Epithelioid Schwannoma: Clinicopathologic Analysis of 63 Cases

Background: Epithelioid schwannoma is rare and may be difficult to recognize. Loss of SMARCB1 expression has been observed in a subset of cases and rare translocations to MPNST have been reported.

Design: 63 cases identified between 2002-2015 were retrieved from consultation files. H&E and immunohistochemical stains were examined. Clinical and follow-up data were obtained from referring pathologists.

Results: Patients were 30 men and 33 women; median age was 44 years (range 13-75). Pathology included: 78% known neurofibromatosis; 3 patients were reported to have multiple lesions. 11 cases were metastatic. 8 cases had bilateral involvement. 5 cases were periosteal. 3 cases were reported to be recurrent. 1 case was positive for S-100 and vimentin. 1 case was negative for S-100 and vimentin. 1 case showed typical morphological features of AH, including a non-lobular, anastomosing pattern of growth.

Imaging studies, available in 13 cases, lacked characteristics sufficient for a diagnosis of AH. In the sole case of a second histologic diagnosis, the AH was noted to show typical morphological features.

Conclusions: AH, originally described in the kidney, also seems to have a predilection for the soft tissues of the paraspinal region. In locations such as these, the diagnosis of AH may be particularly challenging, as imaging studies do not show classical features of hemangioendothelioma, and in tumors in these locations are often sampled with limited needle biopsies. Awareness of the distinctive morphologic features of AH, and appreciation that AH may occur in non-genitourinary sites, should allow its distinct recognition from potentially more aggressive lesions, in particular angiosarcoma.

Follicular Dendritic Cell Sarcomas: Insights into Its Molecular Landscape

Background: Follicular dendritic cell sarcoma (FDCS) is a rare mesenchymal neoplasm arising in intimate association with lymphoid tissue. It is highly sensitive to radiation but may display a broad spectrum of presentations, pathologic phenotypes and clinical behavior. To date, the disease remains poorly understood at the clinical, pathologic and molecular levels. Herein, we investigate the molecular landscape of FDCS and seek candidate drivers for targeted therapies.

Design: DNA from 18 FFPE tumors (16 patients) and matching normal tissue was extracted. Testing was performed by a laboratory developed custom hybridization-capture based assay (MSK-IMPACT) targeting all exons of 410 genes from tumor / normal paired samples. Sequences were sequenced on an Illumina HiSeq 2500 and analyzed with a custom analysis pipeline.

Results: Our series includes 10 male and 6 female patients with a median age of 49 years (range 21-79). Anatomical sites included 4 retroperitoneal, 3 neck lymph nodes, 3 mediastinal, 3 tracheal, 2 liver, 1 colon, 1 chest wall, 1 axilla. A total of 52 mutations involved 42 genes. 3 genes were mutated in >5% cases (c-KIT, KRAS, TP53). 5% of patients harbored 2 or more mutations.

Conclusions: Genetic alterations in tumor suppressor genes rather than activation of oncogenes seem to drive tumorigenesis in FDCS. Loss of PTCH1 and TERT amplification in a subset of cases identify possible candidates for targeted therapies including Hedgehog and Telomerase inhibitors. Reverse transcriptome and whole genome sequencing are warranted to identify potential undetected translocations.

Anatomosing Hemangiomatas Arising in Unusual Locations: A Clinicopathologic Study of 15 Cases Showing a Predilection for the Paraspinal Region

Background: Anatomosing hemangiomata (AH), a recently recognized benign vascular neoplasm originally described in the kidney, may be confused with well-differentiated angiosarcoma owing to its distinctive anastomosing, non-lobular pattern of growth and the presence of minimal endothelial cell atypia. Rare AH have been described in the liver and non-renal genitourinary sites. We report a series of 15 AH occurring in unusual locations, in particular the paravertebral soft tissues.

Design: Descriptive slides from 15 cases of AH were retrieved from our archives and re-reviewed. Clinical, radiographic and follow-up information was obtained.

Results: Cases occurred in 9 M and 6 F (median 67 years of age; range 2 to 85 years). Eleven cases involved the soft tissues near the vertebral column, including cases described as para-vertebral (n=4), posa (n=2), costovertebral angle (n=2), para-aortic, para-sacral, and retroperitoneum (n=1 each). Other locations included the anterior mediastinum, uterine cornu, infundibular pelvic ligament, and upper arm (n=1 each).

Imaging studies, available in 13 cases, showed characteristic findings of hemangioendothelioma. The tumors ranged from 1.5 – 7.5 cm (median, 3.6 cm). All cases showed typical morphological features of AH, including a non-lobular, anastomosing proliferation of capillary-sized vessels with mild endothelial cell nuclear variability, and with the potential shared genetic abnormalities in CCSK and soft tissue URCS. CCSK are characterized either by BCOR exon 16 ITD in most cases or by YWHAE-NUTMB2 fusions in 12% of cases.

Design: Among the 20 infantile URCS selected, 18 showed no known gene fusions, representing the study group. RNA sequencing was applied on 5 URCS with frozen tissue, the remaining being investigated for YWHAE-NUTMB2 by FISH, and DNA PCR for BCOR ITD. A control group including: 3 CCSK, 6 fusion-negative URCS in older children or adults, and 5 cases of other types of infantile sarcomas, was tested for BCOR ITD and YWHAE abnormalities.

Results: The index case was validated for YWHAE-NUTMB2 fusion by FISH and RT-PCR, while lacking BCOR ITD. A 2nd identical YWHAE-NUTMB2 fusion was found in a back-up lesion from a 5 month old boy. The remaining 16 cases and control group lacked YWHAE gene rearrangements; instead BCOR ITD were found in 7/18 (39%) infantile URCS tested. In the control cohort, BCOR ITD was found only in the 3 CCSK, while
absent in all others. Histologically, URCS with both genotypes had similar appearance
with CCSK, composed of poorly differentiated blue round cells with fine chromatin, occasional
orbes and prominent capillary network. The anatomic distribution included trunk (4),
abdomen (3) and H&N (2). RNASeq showed BCR/IR overexpression in BCR-IRD+
positive cases compared to other URCS.

**Conclusions:** Most BCR IR exon 16 IGD (39%) and \(22L\)-\(U\)-NUMB2 fusions (12%) in infantile soft tissue URCS, but not in other subtypes or URCS of older children/ 
adolescents. These findings suggest that a subset of infantile URCS show overlapping
features with CCSS, such as age, histologic features and genetic signature. Based on
this close pathogenetic link with CCSS, these URCS most likely represent the soft
tissue counterparts of CCSK.

66 TLE1 mRNA Chromogenic In Situ Hybridization (CISH): A Potentially Powerful Diagnostic Tool for Synovial Sarcoma

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**Background:** Synovial sarcoma (SS) can be diagnosed by identifying its specific T(8;17)
(SS18-SSX1-2) translocation with resultant SS18-SSX gene fusion by conventional
cytogenetics, fluorescence in situ hybridization (FISH) or polymerase chain reaction
(PCR). However, these methodologies are costly, labor intensive and aren’t available in
all institutions. TLE1 was originally identified as strongly and differentially expressed
in SS. Initial studies using TLE1 immunohistochemistry (IHC) as a surrogate were
promising. However, due to technical issues, TLE1 IHC is no longer reliable. To better
harness the diagnostic power of TLE1 in the diagnosis of SS, we evaluated the utility of
TLE1 RNA CISH.

**Design:** We evaluated 40 cases for TLE1 by mRNA CISH: SS(n=27; 19 MSS, 3 BSS, 5 PDSS),malignant peripheral nerve sheath tumor (MNST) (n=7), dermatofibrosarcoma protuberans (DFSP) (n=4), and benign fibrous histiocytoma (BFH) (n=2). Twenty-one
SS cases had confirmed SS18 translocation. FFPE tissue sections were stained on Venta
Discovery XT automation system using TLE1 and control probes from Advanced Cell
Diagnosics (Heyward, CA). TLE1 signal intensity was scored as strong, weak and
negative, and the distribution was defined as diffuse (>50%), patchy (25-50%), focal
(5-25%) and negative (<5%).

**Results:** All SS cases were positive for TLE1 RNA CISH while all cases of MNST,
DFSP & BFH were negative. Most SS exhibited strong and diffuse positivity, 81% and
70%, respectively. Rare focal (1/27, 4%) and weak (5/27, 19%) positivity was also
observed. Variable heterogeneous TLE1 expression was present in 50% of cases
(15/27), more frequently in BSS (3/3, 100%) and PDSS (4/5, 80%) compared to MSS
(0/19, 42%).

**Conclusions:** Preliminary results show TLE1 mRNA CISH to be a very useful diagnostic
tool for SS with 100% sensitivity and specificity. The quick turnaround time and ease of
interpretation with the ability to visualize the results by conventional light microscopy
make RNAISH comparable with IHC for ease of use. RNAISH CISH is a viable alternative
for diagnostic markers where suitable commercial antibodies may not be available.

67 Spindle Cell/Pleomorphic Lipomas of the Distal Extremities: Not Just a Myth

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**Background:** Spindle cell/pleomorphic lipomas (SC/PLs) typically occur in older
men and are often limited to the posterior neck/back area, including the shoulders.
Cases outside this region, excluding dermal spindle cell lipomas, are exceedingly rare.
We examined lesions classified as SC/PLs in men that arose in the distal extremities.

**Design:** Eight cases of histologically confirmed SC/PLs and located distally to the
elbows and waist line were immunohistochemically evaluated for CD34, p16, Rb1,
desmin, ER and p16. Nuclear Rb1 staining was scored as retained (>75% +), mosaic
(25-75%) and lost (<25%). Other markers were scored as diffuse (D+>50%), patchy
(P+>25 to 50%), focal (F+>25 to 50%) and negative (<5%).

**Results:** Patients ranged from 36 to 80 years of age (median 57) with a median
tumor size of 5.1 cm. Most cases (6/8, 75%) were subcutaneous, 2 of which involved the
subcutaneous tissue. The 2 exclusively subcutaneous cases were both from the thigh.
Myxoid stroma was seen in 88% (7/8) of cases. One PL (8) had pleomorphic vessels
and multinucleated lipoblasts. All cases were positive for CD34. Rb1 was lost in 88%
(7/8) of cases; one case had a mosaic pattern of staining. P16 positivity was present
in 75% of cases. All cases were negative for desmin and ER. Five of 5 cases tested were
negative for MDM2 amplification. The remaining cases had follow-up data ranging
from 122 to 158 months and none recurred.

**Conclusions:** SC/PLs of the distal extremities share similar clinicopathological
characteristics with cases from the posterior neck region, with the exception of being
depth deep. Diagnosis of SC/PLs of the extremities should be made cautiously
and only after excluding atypical lipomatous tumor. P16 does not discriminate SC/
PLs from ALTs.

68 Man Versus FISH: How Accurately Can Soft Tissue Experts Predict MDM2 Amplification of Deep Seated Adipocytic Neoplasms Based on Histology?

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**Background:** Differentiating benign lipomatous tumors (BLT) (lipomas, hibernomas,
etc) from atypical lipomatous tumors/well-differentiated liposarcomas (ALT/WDLPS)
has become substantially easier with the aid of MDM2 fluorescence in situ hybridization
(FISH) to detect MDM2 gene amplification, the defining molecular signature of ALT/
WDLPS. In this study, we sought to determine in a subspecialty consultation setting
whether histology was sufficient to accurately diagnose ALT/WDLPS without the need
to test for MDM2 amplification.

**Design:** Three soft tissue pathologists (SBD, JRG & BPK) and their fellow (VK)
evaluated deep seated adipocytic neoplasms prospectively using only hematoxylin and
cosin stained sides and a diagnosis was rendered. All cases were subsequently tested
for MDM2 gene amplification by FISH. Clinical parameters, including age, sex, site
and size were made available to each reviewer. Histological features used to diagnose
ALT/WDLPS were variation in adipocyte size, hyperchromatic adipocytic nuclei,
and cellular fibrous areas and blood vessel walls with atypical spindle stromal cells.

**Results:** There were 19 cases; 8 ALT/WDLPS in 5 men and 3 women ranging from
38 to 90 years of age (median 65) with a median tumor size of 19 cm, and 11 benign
adipocytic tumors in 5 men and 6 women ranging from 40 to 78 years of age (median
48) with a median tumor size of 7.5 cm. Most of the retroperitoneal tumors were ALT/
WDLPS (75%, 3/4) compared to 36% (5/14) from the extremities. The majority of
BLTs consisted of lipomas with fat necrosis (7/11, 64%) followed by intramuscular
lipomas (2); a lipoma and a hibernoma. On average, attending pathologists had a 93%
accuracy rate of predicating MDM2 amplification; they were more likely to overcall
(10%) than undercall (5%) ALT/WDLPS. The follow up had an 84% accuracy rate with
more undercalling than overcalling, 11% and 5%, respectively. A total of 4 cases, 2
ALT/WDLPS and 2 lipomas were incorrectly interpreted, all of which had significant
fat necrosis; one of the cases was an endobronchial lipoma with extensive fibrosis.

**Conclusions:** Even in the hands of expert soft tissue pathologists, histology comes
close but does not match the accuracy of MDM2 gene amplification. Fat necrosis is
the main culprit for human errors, which can be seen in both ALT/WDLPS and BLTs
and often led to overcalling lipomas as ALT/WDLPS. For the time being, it appears
that it is still necessary to test most, if not all deep-seated adipocytic tumors with
MDM2 FISH.

69 RNA-seq Study of TFE3 Translocation-Associated Perivascular Epithelioid Cell Neoplasm (PEComa)

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**Background:** Perivascular epithelioid cell neoplasms (PEComa) are a family of rare
neoplasms with unknown cell origin. Several studies have reported small
portion of those cases associated with TFE3 rearrangement. Previously, we successfully
identified TFE3 fusion by RNA-seq from formalin fixed paraffin embedded (FFPE)
tissue. Here, we would like to apply this massively parallel sequencing technology to
study TFE3 translocation-associated PEComa.

**Design:** 4 cases of TFE3 translocation-associated PEComa (confirmed by FISH)
were collected for RNA-seq. Total RNA was extracted from FFPE tissue, using the
AllPrep DNA/RNA FFPE. 100 ng RNA was applied for sequencing library preparation using
the TruSeq RNA Access Library Prep Kit as per the manufacturer’s protocol. Paired-end
sequencing (75 bp+2) was performed using the MiSeq Reagent V3 Kit (150 cycles)
and the MiSeq sequencing system. STAR algorithm was employed for detection of
differential TFE3, or other fusion. Bowtie2 was employed for alignment and
counting of short sequence reads to the human genome reference hg19 and the fusion
transcript SPQPSF/PSQ-FTE3. Integrative Genomics Viewer (IGV) was employed for
data visualization.

**Results:** We identified 3 cases with PSF-TFE3 fusion, one case with FOXO1-MTFP1
fusion.
72 Chondroblastoma of Extracranial Bones - Analyses of 103 Cases by Numerical Scoring

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Background: Chondroblastoma (CB) is a rare chondrogenic tumor and categorized as a locally aggressive, rarely metastasizing intermediate bone tumor. No reliable clinicopathologic parameters predicting the local recurrence and/or very rare pulmonary metastasis has been elucidated.

Design: Clinicopathological profiles of 103 cases of histologically proven CB of extracranial bones were reviewed. Radiological images and pathological slides were reviewed. For each case, 10 pathological and 5 radiological features were evaluated by numerical scoring and were analyzed statistically in terms of prognostic significance.

Results: Of 103 cases, age of the patients was ranged 8-61 (average: 19.6 years) with 80 male and 23 female. Frequently involved sites were femur (43.7%), tibia (14.0%), calcaneus (12.6%), patella and humerus (9.7%). Radiologically, tumor were 2-80 (average: 31.1) mm in size, with marginal sclerosis (94.3%) and calcification (55.9%). Histologically, there were immature pink cartilage (91.4%), mitotic figures (44.1%), and chicken wire calcification (32.3%). Current age was usually selected for the initial surgery (91.2%). For the follow-up period ranging 2-260 (average: 53.5) months, local recurrence was noted in 16 cases (15.5%), with period since the initial surgery till the recurrence ranging 4-53 (average: 20.2) months. No patient had metastasis. Recurrent tumors were femur (37.5%), tibia (20.9%), humerus (18.9%), patella, and radius (6.2%). By univariate analysis, only cystic change in radiological image was statistically significant for differentiating recurrent cases from non-recurrent cases. Age of the patients with recurrence was statistically younger than that of the patients without recurrence. Other features, such as sex, tumor size, location, mitotic figures, surgical methods, etc. were insignificant. In multivariate analysis, we could not find any features which predict local recurrence.

Conclusions: In this study, we could not find the clinicopathological features predicting the local recurrence. Local recurrence rate was similar to the previous reports (5-25%), but metastatizing case was not noted. CB is categorized into "rarely metastasizing tumor" which can metastasize in less than 2%, but the current data shows metastasis of extracranial CB is extremely rare.

73 ETV4 is a Useful Marker for the Diagnosis of CIC-DUX4 Round Cell Sarcomas: A Study of 110 Cases Including Mimicking Lesions

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Background: A subset of primitive round cell sarcomas (RCS) remains difficult to diagnose and classify. Among these, a rare RCS harboring a novel gene fusion, CIC-DUX4, has been described. Due to its aggressive clinical behavior and potential therapeutic implications, accurate identification of this novel soft tissue sarcoma is necessary. Definitive diagnosis requires molecular confirmation but yet only few centers can perform this test. Based on several studies that CIC-DUX4 RCS show upregulation of PEA3 subfamily genes (belonging to ETS/transcription factors family) and notably ETV4, we performed a detailed immunohistochemical analysis to investigate the expression of ETV4 among CIC-DUX4 RCS and their potential mimics (especially Ewing sarcomas).

Design: The study cohort included 15 cases of CIC-DUX4 RCS and 95 tumors that could mimic CIC-DUX4 RCS morphologically: 40 Ewing sarcomas (EWSR1 rearrangement detected by Fluorescent In Situ Hybridization (FISH), 25 alveolar rhabdomyosarcoma (FKHR rearrangement detected by FISH), 20 poor differentiated, round cell synovial sarcoma (SS18 rearrangement detected by FISH) and 10 desmoplastic round cell sarcomas (WT1-EWSR1 rearrangement detected by Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR)).

Results: All CIC-DUX4 RCS (on core needle biopsies and open biopsies) showed strong and diffuse ETV4 nuclear positivity.

Among other 95 tumors, only six cases (4 Ewing sarcoma, one alveolar rhabdomyosarcoma and one desmoplastic round cell tumors) showed focal (<5% of tumors cells) and very weak nuclear expression of ETV4; and all other tumors (on core needle biopsies and open biopsies) were completely negative for ETV4.

Conclusions: ETV4 is a useful diagnostic marker in CIC-DUX4 round cell sarcoma. Faced with histologic diagnosis of round cell undifferentiated soft tissue sarcomas that lack molecular evidence of a known sarcoma-associated translocation, a systematic
immunohistochemical evaluation of ETV4, even on core needle biopsies, allows a valuable selection of cases for FISH analysis permitting the definitive diagnosis of CIC-DUX4 sarcoma.

74 Malignant Peripheral Nerve Sheath Tumor Is a Challenging Diagnosis: A Systematic Pathology Review, Immunohistochemistry and Molecular Analysis in 160 Patients from the French Sarcoma Group Database

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Background: An accurate histopathologic diagnosis is essential for an adequate treatment of soft tissue sarcomas. The diagnosis of malignant peripheral nerve sheath tumor (MPNST) can be complex, particularly outside the neurofibromatosis type 1 (NF1) context. MPNST is a rare malignancy and due to the lack of specific histological criteria, a number of non-MPNSTs were placed incorrectly in this category over the years and prognostic factors remain controversial.

Design: Three hundred and fifty patients diagnosed with MPNST (from 1990 to 2013) were retrieved from the French sarcoma network (http://www.treps.sarcomabcb.org) and the Conticabase (http://www.conticabase.sarcomabcb.org). Tumor samples were available for 160 cases (45.2%). Pathology review, immunohistochemistry and molecular analysis (when dealing with a monomorphic sarcoma) suggested of simply 100 cases, which were systematically performed. Patient, tumor, and treatment characteristics were evaluated to identify prognostic factors for the undeniable primary MPNST (n=106) cohort.

Results: Twenty-two (18.3%) initially diagnosed as MPNST were reclassified. Patients with NF1 disease comprised 64% of the remaining cohort. The 5-year overall survival for patients from the entire cohort was 46.96%, 34.78% for NF1 patients and 68.54% for patients without NF1 disease, making NF1 syndrome an independent poor prognostic factor of survival. Patients with NF1 mutation genotypes were independent predictors of local recurrence. Tumor grade (FNCLCC (Fédération Nationale des Centres de Lutte Contre le Cancer) grading system) was an independent prognostic indicator of metastasis.

Conclusions: Given the therapeutic implications of this misdiagnosis, the systematic pathology review, immunohistochemistry and molecular analysis (when dealing with a monomorphic sarcoma) strategy allowed reclassification of 20% of cases, mainly in the subtype MPNST. We, therefore, propose it as a standard management procedure for all sarcomas. Patients with MPNSTs shared prognostic factors similar to those observed in patients with other soft tissue sarcomas. A deeper understanding of NF1-associated MPNST is needed to allow the identification of new treatment strategies.

75 Transcriptional Reappraisal Identifies Overexpressed PLCB4 as an Adverse Prognosticator in Primary Localized Gastrointestinal Stromal Tumors (GISTs)

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Background: In the post-imatinib era, accurate prognostication of GISTs is a critical issue for counseling and identifying other targetable molecules because of inevitable imatinib resistance. Despite being a cancer hallmark of renewed interest, deregulated cellular metabolism remains barely elucidated in GISTs, especially in the arena of lipid bioprocessing.

Design: Through data mining of published transcriptomes (GES8167), we sought for lipid metabolism-regulating metabolic drivers differentially upregulated in high-risk cases and identified PLCB4 (phospholipase C beta 4) as a top-ranking candidate relevant to GIST progression. PLCB4 expression status was validated in 3 GIST cell lines and two independent cohorts of formalin-fixed primary localized GISTs. Of these, PLCB4 mRNA abundance measured by Quantigene assay was informative in 70 cases, and immunoreexpression level informative by H-score method in another 350 cases on tissue microarrays, including 213 cases with known KIT/PDGFBR mutation genotypes. The obtained data were correlated with clinicopathological and KIT/PDGFRB genotypic variables and disease-free survival (DFS).

Results: Both imatinib-sensitive GIST882 cells and imatinib-resistant GIST48 cells exhibited increased expression of PLCB4 mRNA and protein, compared with no expression detectable in imatinib-resistant GIST430 cells. PLCB4 mRNA expression significantly increased from adjacent normal tissue to the non-high-risk group (p=0.007) and from non-high-risk group to high-risk GISTs (p<0.0008). PLCB4 protein overexpression was associated with non-gastric location (p=0.022), unfavorable genotypes (p=0.033) and strongly related to increased size, mitosis, and risk level defined by both NIH and NCCN schemes (all p<0.001). Univariate, decreased DFS was strongly correlated with PLCB4 overexpression (p=0.0001), which remained prognostically independent to predict adverse outcome (p=0.001, hazard ratio: 3.075), together with epithelioid histology and higher risk levels.

Conclusions: PLCB4 is a novel lipid catalysing-regulating enzyme closely linked to GIST, given its strong associations with unfavorable clinicopathological and genotypic factors and independent negative prognostic impact.

76 BCOR-CCNB3 Sarcoma of Soft Tissue with Round and Spindle Histology: A Study of 4 Cases Highlights the Pitfall of Mimicking Poorly Differentiated Synovial Sarcoma

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Background: Derived from X-chromosome paracentric inversion, BCOR-CCNB3 fusion is the molecular hallmark of a recently described undifferentiated sarcoma, predominantly occurring in the bones of male adolescents and exhibiting "Ewing-like" histology in some cells. Only 13 molecularly validated cases were reported to primarily originate in the soft tissue, leaving the histological spectrum and tumor behavior largely undefined.

Design: Four undifferentiated sarcomas of soft tissue comprising round and spindle cells were identified from archives. Ewing sarcoma protein (EWS) was identified by immunohistochemistry and by RT-PCR in all cases, whereas BCOR-CCNB3 fusions were ruled out. The prognosis was poor with all patients eventually dying within 2 years of diagnosis.

Conclusions: BCOR-CCNB3 sarcoma is a novel undifferentiated soft tissue malignancy, which shares clinical and histological overlap with other round cell sarcoma types, but which lies distinct. The potential for misdiagnosis exists, given the mimicking potential of this entity with Ewing sarcoma and other round cell sarcomas.
Design: Sarcomas cases included in the radiation oncology database of this institution over the past 15 years were reviewed for this study. Cases with a treatment naïve biopsy and/or adjuvant-treated resection specimen were included. Eighty-five patients met criteria and were used to create multiple tissue microarrays. Two PD-L1 clones (Cell Signaling, E1L3N; Spring BiScience, SP142) and 1 PD-L1 clone (Abcam, NAT105; BioSource, B-A05) were optimized for this study. Cases were considered positive for each clone if greater than 5% staining was detected.

Results: Most cases had both a pre-treatment biopsy and post-treatment resection for analysis. The tumors showed similar staining across all clones. Seventeen of 85 tumors were considered positive for SOST in non-neoplastic bone, and 19 of 85 tumors were considered positive with the known functions of SOST in non-neoplastic bone. In aggregate, these findings are entirely unknown.

Design: Tissue from 117 curettage specimens (51 GCTBs, 38 CBAs and 28 ABCs) were collected from the archives of the Calgary Laboratory Services/University of Calgary (31 cases, 2009–present) and Vanderbilt University Medical Center (86 cases, 1999–2010). Diagnostic cytology specimens were available for 9 patients (8 GCTBs and 1 CBA). PCR was conducted to amplify the region of interest in H3F3A or H3F3B followed by a SNAPshot® to interrogate each nucleotide in H3F3A codon 34 and H3F3B codon 36. All ABCs and 18 ABCs were tested for H3F3A mutations; all ABCs and 10 ABCs were tested for H3F3B mutations.

Results: 36/51 GCTBs (71%) had an H3F3A G34W mutation. 33/38 CBAs (87%) had an H3F3B K36M mutation. Of 33 H3F3A G34W mutations, which may represent a pre-existing GCTB. All 9 pre-curettage cytology specimens showed concordant molecular findings with the subsequent curettage (positive, 8; negative, 1). Of 3 curettage specimens also showed concordant molecular findings (2 positive, 1 negative). Of note, one of the cytology specimens was initially diagnosed as consistent with GCTB, but the diagnosis was changed to CBA upon curettage; both specimens were positive for H3F3B K36M. 3 curettage specimens with indeterminate features (2 favoring CBA, 1 favoring GCTB) were tested for both H3F3A and H3F3B mutations. Each was positive for only one mutation, helping to clarify the diagnosis. These results could prove a useful adjunct to histologic assessment in challenging cases. This is especially true in tumor specimens, where minimal material may preclude a definitive morphologic diagnosis but molecular testing is still possible.

81 Immunohistochemical Profile of Littoral Cell Angioma of the Bone. A Report of 25 Cases Predominantly Associated with Visceral Malignancies

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Background: Littoral cell angioma (LCA) is a rare primary splenic vascular tumor frequently associated with internal malignancies. LCA demonstrates non-specific morphology. Immunohistochemistry (IHC) can prove distinct hybrid endothelial/histioscytic phenotype of littoral cells and helps to render the right diagnosis. We present a group of 25 LCA with a detailed immunohistochemical analysis and an emphasis on their frequent association with visceral malignancies.

Design: 25 original cases of LCA were collected from various institutions. All cases were examined by light microscopy and 17/25 cases with available tissue blocks were immunohistochemically stained for endothelial and histioscytic markers. Clinical data and follow-up were retrieved from respective institutions.

Results: Patients were 16 males, 9 females with age range 32-86 years (mean 56.2). Follow-up was available for 23/25 patients, range 1-21 years (mean 8.6). All tumors had a multinodular cystic appearance with the size of the nodules ranging from 0.2 to 4 cm. Morphology fulfilled current histological criteria for LCA. All cases were positive for H3F3A G34W mutation for GCTBs, and the H3F3B K36M mutation for CBAs. Testing for these mutations could prove a useful adjunct to histologic assessment in challenging cases.

Conclusions: There are many cases of LCA that may represent a pre-existing GCTB. All 9 pre-curettage cytology specimens showed concordant molecular findings with the subsequent curettage (positive, 8; negative, 1). Of 3 curettage specimens also showed concordant molecular findings (2 positive, 1 negative). Of note, one of the cytology specimens was initially diagnosed as consistent with GCTB, but the diagnosis was changed to CBA upon curettage; both specimens were positive for H3F3B K36M. 3 curettage specimens with indeterminate features (2 favoring CBA, 1 favoring GCTB) were tested for both H3F3A and H3F3B mutations. Each was positive for only one mutation, helping to clarify the diagnosis. These results could prove a useful adjunct to histologic assessment in challenging cases. This is especially true in tumor specimens, where minimal material may preclude a definitive morphologic diagnosis but molecular testing is still possible.

82 P16 Expression as a Prognostic and Predictive Marker in Osteosarcoma of Bone

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Background: The potential prognostic and predictive value of p16 (p16INK4a) in high grade osteosarcoma of bone has been recently investigated in small series and the results from different studies were somewhat controversial.

Design: Immunohistochemical analysis of p16 expression was performed in a series of 317 cases of high grade osteosarcoma of bone (grade 3 according to World Health Organization, grade 4 according to Broders system) to explore its potential prognostic...
and predictive value. The main criteria of selection was the availability of adequate material from both the initial biopsy and the post-chemotherapy surgical sample to allow morphologic and immunohistochemical characterization. Immunohistochemistry was performed with a commercially available p66 monoclonal mouse antibody. Follow-up data were available in all cases with a mean of 126 months (range: 6-366 months).

**Results:** p66 was detected in 70.6% (252/357) of cases. A significant association was noted between p66 expression and pathologic complete response to chemotherapy (chi-square test 25.307; P< 0.001); conversely, no association could be established between p66 expression and age, gender, tumor site and histologic subtype. Kaplan-Meier survival analysis demonstrated that the absence of p66 expression was significantly associated with an adverse metastatic disease-free survival (p=0.012), disease-free survival (p=0.04) and overall survival (p=0.05) when compared with the presence of p66 expression. Multivariate Cox regression analysis did not estimate p66 expression to be an independent prognostic and predictive factor (hazard ratio (HR) = 1.422 for metastases free-survival (MFS), p=0.052; HR = 1.288 for disease free-survival (DFS), p=0.181; HR = 1.322 for overall survival (OS), p=0.179) at difference to pathologic response to chemotherapy that represented the only independent prognostic factor in our series (HR = 1.933 for MFS, p=0.001; HR = 2.145 for DFS, p=0.001; HR = 2.395 for OS, p=0.001).

**Our data indicate that:** 1. Immunohistochemical expression of p66 in high-grade osteosarcoma significantly correlates with histologic response to chemotherapy. 2. p66 immunonegativity is associated with worse prognosis although it does not represent an independent prognostic biomarker.

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**83 Exploring Intra-Tumor Heterogeneity in Neurofibromatosis Type 1 Plexiform Neurofibromas: Integration of Histological and Genomic Data Reveals Correlation between Progressive Loss of CDKN2A and Degree of Cellular Atypia**

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**Background:** Plexiform neurofibromas (PNFs) are one of the major complications of patients with Neurofibromatosis type 1 (NF1). PNFs affect more than 40% of NF1 patients and a subgroup of them undergo malignant transformation. Contrary, dermal neurofibromas (DFs) are not associated with an increased risk of malignant transformation.

**Design:** We aimed at exploring the histological and molecular variation within PNFs we histologically characterized 8 plexiform neurofibromas, previously divided in multiple sections. Cellular density, atypia and other morphologic aspects were evaluated.

**Results:** PNFs exhibited a different degree of intra-tumor histological and biological diversity. Genotype integrity of the different PNFs was almost non-altered, with the exception of the NF1 locus and long arm of chromosome 17, where the NF1 gene is located. Cell density and atypia were correlated with molecular genetic findings, and 2/8 cases showed morphological and genetic intra-PNF heterogeneity. In PNF3, sections with higher cellular density and atypia were correlated with the presence of higher levels of NMYC+/CDKN2A- Schwann cells (SC), and CIC-DUX4 fusion followed by poor differentiation (NMYC+/CDKN2A−/−/SC). In PNF4, the increasing degree of atypia exhibited in different sections was correlated with the progressive loss of CDKN2A loci.

**Conclusions:** Our results support the initial hypothesis of intra-tumor heterogeneity within a PNF. Moreover, we identified the progressive loss of CDKN2A as a key feature in affecting tumor density.

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**84 RNA-sequencing Identifies ETV6-NTRK3 as a Gene Fusion Involved in Gastrointestinal Stromal Tumors**

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**Background:** The vast majority of gastrointestinal stromal tumors (GIST) are driven by oncogenic activation of KIT, PDGFRα or, very rarely, BRAF. Loss of succinate dehydrogenase complex activity has been identified in a subset of KIT/PDGFRα/BRaf-mutation negative GIST. Yet, there is a significant fraction of GIST devoid of such alterations. We sought to explore the possible involvement of fusion genes in this “quadruple-negative” subgroup.

**Design:** RNA-sequencing was performed on FFPE samples from 5 quadruple-negative GIST. FusionCatcher, ChimeraScan and a in-house algorithm were used to identify fusion events. RNA-seq and RNA sequencing with paired end sequencing of the RT-PCR products. The tissue case was located in the rectum, featured an epithelioid morphology, showed a strong and diffuse expression of CD117 and DOG1, and fell in the high risk category based on AFIP criteria. No additional ETV6-rearranged cases were identified by FISH in a previously enriched for KIT/PDGFRα/BRaf-mutation negative (4) and rectal cases (4). Intriguingly, similar to what reported for the infantile fibrosarcoma chimeras, also the ETV6-NTRK3 fusion in our index case triggered activation of IGF1R signaling pathway and sensitized HT1080 and U2OS cells to IGF and ALK/MET inhibitors in overall. Our data suggest that the ETV6-NTRK3 fusion might identify a rare subset of GIST with peculiar clinicopathologic characteristics (quadruple negative and rectal location) which could be eligible for unprecedented targeted approaches.

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**85 Histology and Fusion Status in Metastatic Rhabdomyosarcoma**

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**Background:** Recent studies on intermediate risk rhabdomyosarcoma (RMS) highlight the shift in the diagnostic criteria of alveolar RMS (ARMS) which occurred in 1997 and 2007 following publication of the International Classification of Rhabdomyosarcoma. This shift resulted in over-diagnosis of ARMS cases in the Children’s Oncology Group (COG) RMS trials during this period. These studies also confirmed that intermediate risk patients with fusion negative ARMS have an outcome similar to a superior event free survival and metastases free survival compared to either a PAX3 (P13) or PAX7 (P7F) - FOXO1 fusion. The goal of the current study was to assess the impact of this diagnostic shift and the effect on outcome for patients with metastatic disease enrolled on two completed high-risk clinical protocols conducted between the years 1999 and 2010.

**Design:** We conducted a histologic re-review for patients with known fusion status including 82 patients enrolled on the high risk COG RMS study D9802 (1999-2004) and 62 patients enrolled on the subsequent high risk COG study ARST0431 (2006-2010). Approximately 35% of patients enrolled on ARST0431 did not have pathologic material for re-review but did have an original central pathology review diagnosis and were used in the outcome analysis.

**Results:** Approximately 20% of patients (18/82) enrolled on D9802 with an original diagnosis of ARMS were reclassified as ERMS. In contrast, 5% of patients (3/62) enrolled on ARST0431 with an original diagnosis of ARMS were reclassified as ERMS. 5 year event-free survival (EFS) for all patients with high-risk RMS was approximately 25%. However, for patients with confirmed or re-classified ERMS the EFS was 45% vs. 30% for fusion negative ARMS, 20% for patients with P7F and 10% for patients with P3F (p=0.020). When analyzed by fusion status alone, EFS was approximately 40% for patients with fusion negative RMS v. 10% for patients with fusion positive RMS (p=0.001). Patients with ERMS treated on D9802 had an inferior outcome to those treated on ARST0431 (25% vs. 60% EFS, p=0.012).

**Conclusions:** The discordance in diagnostic re-classification for COG study D9802 vs. ARST0431 highlights the over-diagnosis of ARMS for patients enrolled on COG clinical trials between 1997 and 2007. Studies performed after this have reliable central pathology review data. As seen for intermediate risk RMS, patients with high risk fusion negative RMS have a superior outcome than RMS patients with either a P3F or P7F fusion.

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**86 CIC-DUX4 Fusion-Positive Round Cell Sarcomas of Soft Tissue and Bone: Clinicopathologic and Molecular Analysis from a Single Institution**

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**Background:** Round cell sarcomas lacking specific translocations of Ewing sarcoma (EWS) are classified in the current WHO classification as “undifferentiated round cell sarcomas (URCS)” and represent a diagnostic challenge. Recently CIC (gene most often but not exclusively with DUX4) rearrangement has been identified in a subset of URCS. To date fifty-nine cases of CIC-DUX4 positive sarcomas (CDS) have been reported, all arose in soft tissue or rarely in visceral sites.

**Design:** Fifty-one cases of URCS of bone and soft tissue treated in our institution from 1997 to 2014, lacking EWSR1 or FUS gene rearrangement with available clinicopathological, genetic and molecular data were available. All cases were retrospectively analyzed immunohistochemically for CD99 and WT1 and molecularly for the presence of the CIC-DUX4 translocation by quantitative real time RT-PCR (qRT-PCR) analysis.

**Results:** Nineteen cases were not adequate for molecular analysis due to absence of neoplastic cells in the sample; 25 were negative and 7 showed the presence of the CIC-DUX4 chimeric transcript. The presence of CIC gene rearrangement was confirmed by FISH analysis in all cases. Patients’ age ranged from 15 to 44 years (median: 33). Male to female ratio was 1:3.7. All but one case arose primary in the soft tissue. Importantly one case originated from the right acromion, thus representing the first primary bone CDS reported to date. Morphologically, all cases showed an undifferentiated round cell population, arranged in nodules separated by fibrous septa, with abundant geographic necrosis, and occasional myxoid change of the stroma was seen. The tumors were focally positive or negative for CD99, and all but two showed cytoplasmic and/or nuclear immureactivity for WT1.
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87 The Diagnostic Utility of CMA1 and TFE3 Immunohistochemistry in Epithelioid Vascular Tumors
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Background: Epithelioid vascular tumors represent a broad spectrum of mesenchymal lesions that includes benign (epithelioid hemangioendothelium), low grade malignant (epithelioid angiosarcoma) and high grade malignant (epithelioid angiosarcoma). Correct diagnosis is often challenging, however the presence of specific molecular aberrations involving the CMA1 and TFE3 genes has been reported in epithelioid hemangioendothelioma (EHE) and epithelioid sarcoma (E1).

Results: In total, 51 patients, including 34 (67%) with soft tissue and 17 (33%) with bone tumors, were studied. The diagnosis of epithelioid vascular tumor was confirmed in 38 cases, 23 EHE, 9 epithelioid angiosarcoma and 6 epithelioid sarcoma. Immunohistochemistry for CMA1 and TFE3 expression was to be mutually exclusive, and both represent helpful markers in the histopathologic classification of epithelioid vascular tumors.

Conclusions: TFE3 and CMA1 expression is useful for differential diagnosis of epithelioid vascular tumors, and should not be misinterpreted as A VMs.

88 Identifying Unique Genome Abnormalities That Distinguish Enchondroma from Chondrosarcoma
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Background: Distinguishing enchondroma from grade 1 and even grade 2 conventional chondrosarcoma based on histologic features can be a diagnostic challenge, but has strong implications for clinical management. Enchondroma, a benign cartilage neoplasm, is usually cured by simple curettage. In contrast, chondrosarcoma is a malignant neoplasm of bone which tends to locally recur and may metastasize. There remains a need for an ancillary molecular tool to help distinguish enchondroma from chondrosarcoma.

Design: SNP-based cytogenomic microarray analysis (CMA) using the Illumina Infinium CytoSNP-850K platform was performed on genomic DNA isolated from formalin-fixed paraffin-embedded tissue of 5 cases of enchondroma and 8 cases of chondrosarcoma. Chondrosarcomas were characterized by bone invasion/entrapment and aggressive features on imaging. Enchondromas lacked invasive growth and were confined to bone. For each case, a total intensity ratio and allele frequency were generated to represent net copy number changes and regional genetic abnormalities (e.g. aneuploidy, deletion, amplification and loss-of-heterozygosity). To represent the genetic complexity of each case, a genomic index (GI) of (total number of abnormalities/eascane) was generated to represent net copy number changes and regional genetic abnormalities.

Results: All enchondroma cases (5 short bones and 1 long bone; age range: 18-57; sex: 3 F: 2 M) showed no copy number changes or regional genetic abnormalities. By contrast, all chondrosarcoma cases (1 grade 1, 6 grade 2 and 1 grade 3; 1 long bone, 1 vertebral column, 2 short bones, 4 flat bones; age range: 20-77; sex: 5 F: 3 M) demonstrated complex genetic alterations with frequent chromosomal losses. Recurrent chromosomal alterations in at least 50% of cases include losses of 6q and 13 (100%), loss of 1p, 5p, 9p and 11 (83%), loss of 4q, 5p, 9q, 10, and 22 (67%) and loss of 4q, 14q, 16q, and 21 (50%). One metastatic chondrosarcoma was characterized by 5 whole-chromosome losses and 10 partial deletions. Chondrosarcoma cases with the highest GI ranged from 4 to 20 and appeared not to correlate with grade.

Conclusions: SNP-based CMA demonstrates complex copy number and regional genetic alterations, including recurrent loss of 6q and 13, in chondrosarcoma in contrast to no abnormalities in enchondroma. Stratification of genetic complexity by the GI clearly distinguishes enchondroma from chondrosarcoma, but does not differentiate low-grade from high-grade chondrosarcoma. SNP CMA is a potentially useful molecular tool to distinguish enchondroma from chondrosarcoma in diagnostically challenging cases.

89 Loss of H3K27 trimethylation Distinguishes Malignant Peripheral Nerve Sheath Tumors from Histologic Mimics
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Background: The differential diagnostic diagnosis of malignant peripheral nerve sheath tumor (MPNST) is challenging, particularly in the sporadic setting and in the absence of supportive markers. Inactivation of the ploidy repress complex (PRC) 2, resulting from inactivating mutations of its constituents SUZ12 or EED, has recently been identified in 70-90% of MPNST. Homozygous PRC2 inactivation results in loss of histone H3K27 trimethylation (H3K27me3). PRC2 inactivation promotes tumor progression and may render patients sensitive to epigenetic-based targeted therapies. H3K27me3 loss has not yet been validated as a diagnostic marker of MPNST.

Design: We performed immunohistochemistry using a rabbit monoclonal antibody directed against trimethylated lysine 27 of histone H3 (1:50 dilution; clone 07-449; Millipore) in 100 MPNST (70 sporadic; 10 neurofibromatosis type 1 (NF1); 10 radiation-associated, 10 epithelioid; 31 low, 36 intermediate, 33 high grade) and 200 other benign and malignant spindle cell neoplasms that represent potential mimics (2 each monosynphonic sarcoma, leiomyosarcoma, dedifferentiated liposarcoma, malignant solitary fibrous tumor, low-grade fibromyxoid sarcoma, fibromatosis, schwannoma, spindle cell melanoma, unclassified post-radiation sarcoma; 10 atypical neurofibroma, spindle cell rhabdomyosarcoma, gastrointestinal stromal tumor, hemangiopericytoma, dermatofibrosarcoma protuberans).

Results: In total, 51 (51%) MPNST, including 34 (49%) sporadic, 7 (7%) NF1-associated, 10 (100%) radiation-associated, and no epithelioid MPNST were negative for H3K27me3. An additional 6 (6%) MPNST showed heterogeneous H3K27me3 expression. Among the 90 sporadic, NF1-associated, and radiation-associated MPNST, complete H3K27me3 loss was observed in 29% of low grade, 59% of intermediate grade, and 83% of high grade tumors (low vs. intermediate/high grade, P < 0.0003). Among other tumor types, 4 (20%) unclassified post-radiation sarcomas were negative for H3K27me3, whereas all other neoplasms were positive.

Conclusions: Loss of H3K27me3 is highly specific for MPNST (although only modestly more sensitive than S-100 protein and SOX10) and may be a useful diagnostic immunohistochemical marker. Our findings suggest that PRC2 inactivation in MPNST may occur during progression to higher grades. Detection of H3K27me3 loss in previously unclassified radiation-associated sarcomas indicates that a subset of these tumors may represent MPNST. The value of H3K27me3 as a predictive marker for sensitivity to epigenetic-based therapies remains to be determined.

90 Non-syndromic Low-Flow Mixed Venous/Lymphatic Malformation of Skeletal Muscle of the Extremity (Fibro-Adipose Vascular Anomaly): A Clinicopathologic Study of 11 Cases with Emphasis on Distinctive Histopathologic Features
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Background: Non-syndromic low-flow vascular malformations of skeletal muscle are often misdiagnosed as cavernous hemangiomas or arteriovenous malformations (AVMs), which may impact management. Recently, a subtype of intramuscular mixed venous/lymphatic malformation was described and termed “fibro-adipose vascular anomaly” (FAVA). Here we report 11 new cases consistent with this lesion and emphasize its distinct morphologic features and prominent lymphatic component.

Design: Cases diagnosed as cavernous hemangioma or malformation of skeletal muscle of the limb were retrieved and histologic features re-evaluated in conjunction with a vascular anomaly expert. Clinical, imaging, operative and follow-up data were obtained. Cases with high-flow imaging characteristics or history of PTEN/other syndromes were excluded. D2-40 immunostain and elastic stain were performed. Nine ordinary intramuscular venous malformations (VMs) were studied for comparison.

Results: Eleven patients (9F, 2M), median age 21 years (range 6-50), presented with significant pain (9/11, duration 6 mo-6 years). None had contractions. Locations included deep muscle of the calf (5), distal thigh (3), forearm (2) and foot (1). All cases were FM VM with clusters of thin “honeycomb” veins and fibrofatty replace (11/11), conspicuous abnormal lymphatics confirmed by D2-40 immunostain (9/10), lymphoid aggregates (10/11), and hemosiderin deposits (9/11). Eight cases showed distinctive indeterminate vessels with concentric hypertrophy and narrowed lumina resembling lymphatics with absent/interrupted internal elastica. Two cases had osification. Venous thrombi were rare (2/11). In contrast, ordinary VMs showed large collapsed veins with frequent thrombi (9/9) and rare “honeycomb” veins (2/9), lymphoid aggregates (19/19) and focal lymphatics (2/9). Fibrosis was present in 2/9 and hemosiderin in 4/9 ordinary VM. Indeterminate vessels were suggested only focally in one case. Six of 9 ordinary VMs were painful, indicating that the latter is not a reliable criterion to separate these lesions.

Conclusions: Non-syndromic intramuscular mixed venous/lymphatic malformations, for which the designation “FAVA” has been suggested, has distinctive clinic- and histopathologic features which set it apart from ordinary intramuscular VMs. The distinctive indeterminate vessels resemble arteries; however, these are low-flow lesions and should not be misinterpreted as AVMs.

91 SIRT5 is Required for Viability of the Ewing Sarcoma Cell Line A673 and the Osteosarcoma Cell Line U2OS
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Background: Siruins are a diverse family of enzymes possessing a range of biological functions, biochemical activities, and protein targets. While best known as deacetylases with roles in lifespan extension, the extent of their impact on metabolic pathways, functions to modulate PDC activity and should not be misinterpreted as A VMs.
Functional suppression of PDC activity is a relatively common event in cancer and is an established mechanism that contributes to metabolic reprogramming. In our current work, we investigated whether SIRT5 is required for viability in two sarcoma cell lines.

**Design:** We used a lentiviral vector system to deliver shRNAs targeting SIRT5 in the U2OS and A673 cell lines. Cell viability was quantified over time with the WST-1 spectrophotometric assay. All experiments were accompanied by parallel controls, including cells infected by non-targeting shRNAs and uninfected control groups. Statistical analyses were completed using the t-test.

**Results:** shRNA mediated knock-down of SIRT5 results in a dramatic, rapid decrease in cell viability relative to controls in both the U2OS and A673 cell lines (p<0.0001).

**Conclusions:** Our preliminary data support the hypothesis that SIRT5 is required for viability in U2OS and A673 sarcoma cell lines. We hypothesize the mechanism involves metabolic reprogramming mediated by SIRT5 deacetylation. Specifically, we believe SIRT5-mediated deacetylation of the E1 subunit represents an important regulatory switch that inactivates the PDC and suppresses mitochondrial respiration, thereby providing crucial precursors for macromolecular synthesis, without which the cells die. As a result, SIRT5 may represent a therapeutic target for the treatment of soft tissue sarcomas.

**Effects to identify compounds that inhibit SIRT5 enzymatic activity are ongoing.**

92 Leiomysarcomas and Rhabdomyosarcomas Over-Express Nicotinamide Phosphoribosyltransferase

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**Background:** Nicotinamide Phosphoribosyltransferase (Nampt) is over-expressed in a variety of malignancies, including ovarian, gastric, thyroid, colorectal, esophageal, breast, and many soft tissue and neural tumors. Nampt catalyzes the rate-limiting step of nicotinamide adenine dinucleotide synthesis (NAD) promoting cell growth and division.

**Design:** To microarrays (TMAs) were purchased form US Biomax, which collectively had approximately 60 benign smooth and skeletal muscle, and leiomyosarcoma, leiomyoma and rhabdomyosarcoma samples from different patients. The TMAs were subject to IHC analysis. The mouse primary monoclonal anti-Nampt antibody (#ALX-804-717, Plymouth Meeting, PA) was used, with a secondary anti-mouse antibody. Relative Nampt protein IHC expression was determined as the product of immunostain intensity and percent of cells stained. Both were scored on a 0-3 scale, with 3 being maximal. Immunostain intensity was scored with no staining being 0, light staining as 1, moderate staining as 2, and heavy staining as 3. The percent of cells stained was measured with no detectable staining as 0, 1-33% as 1, 34-66% as 2, and 67-100% as 3.

**Results:** Nampt levels were very low in benign smooth and skeletal muscle, slightly elevated in benign leiomyosomas, and significantly elevated in leiomyosarcomas and rhabdomyosarcomas. Interestingly, Nampt levels were higher in high-grade leiomyosarcomas compared to low-grade. Additionally differences in Nampt staining were found between different rhabdomyosarcoma subtypes.

**Conclusions:** Nampt protein levels are elevated in several different malignancies, with some data indicating that higher Nampt levels correlate with a worse prognosis, chemotherapy resistance, and a greater likelihood for metastasis and local invasion. Here we show that Nampt levels are increased in leiomyosarcomas and rhabdomyosarcomas, with the grade of leiomyosarcoma and rhabdomyosarcoma subtype correlating with specific Nampt expression levels. Nampt activity is necessary for NAD production and hence tumor growth and division. Our preliminary data support the hypothesis that Nampt is required for viability in leiomyosarcomas and rhabdomyosarcomas.

93 Single Institutional Analysis of Prognostic Factors for Soft Tissue Sarcoma Measurable Volume of Tumor Depth

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**Background:** Histologic grade, tumor size, tumor depth, nodal involvement, remote metastasis are prognostic factors of soft tissue sarcoma. These factors are included in AJCC/UICC staging system. But its value has been reconsidered. Tumor depth is a confound factor related to tumor size. In our current study, we examined the significance of tumor depth in univariate analysis.

**Results:** In univariate analysis, Histologic grade (Grade2: Relative risk (RR)=8.514 p<0.001; Grade3: RR=17.515 p<0.001) showed a good prognostic factors. Histologic grade and tumor size were independent factors (p=0.217) was not. In chi-square test, size and depth were significantly related (p<0.001).

**Conclusions:** These results suggest that tumor depth is a confound factor related to tumor size. As a result, SIRT5 may represent a therapeutic target for the treatment of soft tissue sarcomas. Efforts to identify compounds that inhibit SIRT5 enzymatic activity are ongoing.

94 Expression of Enhancer of Zeste Homolog 2 (EZH2) Protein and Histone H3 Lysine 27 Trimethylation (H3K27me3) in Histologic Grade and Depth of Tumor Cell Line and Neoplasms

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**Background:** EZH2, a member of the polycomb protein group, is an important methyltransferase that is over-expressed in various carcinomas and hematopoetic neoplasms. We have investigated EZH2 expression in the range of histiocytic and dendritic cell neoplasms and correlated its expression with that of p-ERK, MYC, and p-STAT3, potential regulators of EZH2 expression.

**Design:** Immunohistochemical staining (IHC) for EZH2 was performed on 63 cases of histiocytic and dendritic cell neoplasms. Immunostaining with anti-EZH2 (IHC in normal skin) showed smooth muscle, benign leiomyomas, leiomyosarcomas, and rhabdomyosarcomas.

**Results:** EZH2 was present in 60% of cases, including 38% of histiocytic / lymphoid neoplasms, 31% of histiocytic sarcoma, 63% of myeloid / plasmacytoid dendritic cell neoplasms (PDC), 12% of histiocytic sarcoma (HS), 17% of follicular dendritic cell sarcoma (FDSC), 15% of Langerhan cell histiocytosis (LCH), and 12% of dendritic cell sarcoma (DCS). These results suggest that EZH2 expression is not limited to myeloid / plasmacytoid dendritic cell neoplasms and may be present in other histiocytic and lymphoid neoplasms.

**Conclusions:** EZH2 expression is not limited to myeloid / plasmacytoid dendritic cell neoplasms and may be present in other histiocytic and lymphoid neoplasms. These findings further support the hypothesis that EZH2 expression is a prognostic marker for these neoplasms. Further studies are needed to determine if EZH2 expression is an independent predictor of survival in these neoplasms.
Conclusions: The NGS panel is a promising tool in identifying fusions in BST cases as well as the expected results in 69% of the informative tested cases. Further optimization, particularly with regards to fixation and RNA extraction may improve the sensitivity of this assay. Although not without its limits, this panel is a potentially powerful tool in difficult to diagnose cases or cases with limited tumor cells.

96 Mass Spectrometry Highlights Proteomic and Epigenetic Changes upon PR2 Loss in MPNST
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Background: The polyclomb recombinator complex 2 (PR2) is a key epigenetic regulator and its dysfunction has been implicated in human malignancies. Recent work has established central role for loss-of-function mutations in PR2 component in the pathogenesis of MPNST. We have developed techniques to apply proteomics approaches to the study of FFPE tissue samples. We present a quantitative proteomic approach to the investigation of protein expression and histone post-translational modifications (PTMs) in a set of MPNST from our pathology archives.
Design: Neurofibromas and MPNST in patients with documented NF1 were selected following histologic review. Whole tissue sections of H&E as well as IHC stains for H3K27me3, desmin and myogenin were reviewed. Total cellular protein was isolated from FFPE tissue cores, digested and analyzed using nano-hpliquid chromatography and tandem mass spectrometry (LC-MS/MS). Histones were isolated by gel electrophoresis, in-gel digested and purified using LC-MS/MS. Histone and PTM quantification were performed using lab-developed software. Statistical analysis was performed using Student’s t-test and hypergeometric distribution of gene ontology annotations (DAVID and GOrilla software tools).
Results: 4/8 MPNSTs showed complete loss of H3K27me3 by immunohistochemistry and histone analysis. This included a complete loss of H3K27me3 in all four cases classified at triton tumors. Direct comparison of histone PTMs in an NF and an MPNST from the same patient revealed significant decreases in the histone marks H3K27me2, H3K9me3 and H3K9me2 in MPNST, all associated with gene repression. Comparative proteomic analysis of MPNSTs with and without the gene rearrangement other than H3K27me3 loss using hypergeometric distribution of gene ontology annotations showed increased expression of proteins involved in nucleosome and chromatid remodeling in tumors with loss of PR2 activity.
Conclusions: Mass spectrometry reliably detects and quantifies global changes in histone modifications correlated with PR2 loss of function in archived tumors. The loss of H3K27me3 was associated with an increase in proteins involved in chromatin and nucleosome reorganization, suggesting that PR2 loss leads to altered genome organization in a subset of MPNSTs. Interestingly, triton tumors all showed H3K27me3 loss, suggesting that PR2 loss facilitates divergent skeletal muscle differentiation.

97 ETV6 Gene Rearrangement in Inflammatory Myofibroblastic Tumor
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Background: The aim of this study was to elucidate the pathological features of inflammatory myofibroblastic tumor (IMT) with the gene rearrangement other than ALK. Design: We investigated ALK, ROS1, ETV6, NTRK3 and RET in 36 cases of IMT using immunohistochemical staining (IHC), fluorescence in situ hybridization (FISH) and reverse transcription-polymerase chain reaction (RT-PCR).
Results: ALK IHC and ROS1 IHC were positive in 22/36 (61.1%) and 2/36 (5.6%) cases, respectively. One case with ROS1 positivity also showed the ROS1 expression by IHC and the TFG-ROS FISH fusion transcript by RT-PCR. Two cases of pulmonary IMT, in a 7-year-old and a 23-year-old patient, had ETV6 gene rearrangement, and the ETV6-NTRK3 fusion transcript was confirmed in one case. These tumors were composed of biphasic myxoid and highly cellular areas with rich plasmacytic infiltration; the histological features were different from those of infantile fibrosarcoma. RET gene rearrangement was not detected.
Conclusions: These results suggest that a subset of ALK-negative IMTs have the gene rearrangement of ROS1, ETV6 or NTRK3 as a possible oncogenic mechanism, and that the detection of these alterations may be of diagnostic value and helpful for determining promising therapeutic strategies.

98 Establishment and Gene Expression Analysis of a Mouse Model for CIC-DUX4 Sarcoma
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Background: CIC-DUX4 fusion gene was identified from soft parts using a retrovirus vector. The BALB/c mouse was subcutaneously transplanted with the infected cells. Gene expression profiles of CIC-DUX4 sarcoma (CDS) were analyzed by cDNA microarray and compared to those of the Ewing sarcoma model. Inhibitory drugs were screened using CDS inhibitory hit kit.
Results: All the transplanted mice developed a solid tumor mass within 4 weeks. The tumor consisted of aggressive proliferation of HA-positive short spindle cells. Comparisons of gene expression profiles between CDS and ES, or between CDS and normal tissues indicated significantly up-regulated genes including Envl, Env, Vgfr, Crk and Zic1, known targets of CIC-DUX4. GSEA showed enrichment of gene sets of characteristic pathways such as cell cycle, adhesion and VEGF signaling in CDS. Immunohistochemistry revealed the distinctive expression of some molecules in CDS comparing with ES, identifying potential biomarkers for diagnosis of CDS. Several specific drugs showing efficient inhibitory effects for CDS cells were identified.
Conclusions: We successfully established a CIC-DUX4 fusion mouse sarcoma model and these results underscore usefulness of the mouse model and distinct biological features of CDS.

99 Expression of TLE-1 and CD99 in Carcinoma: Pitfalls in Diagnosis of Synovial Sarcoma
Daniel Zaccarini, Xiaohong Dong, Jamie Tall, Charlene Maciak, Alfredo Valente, Shengle Zhang. SUNY Upstate Medical University, Syracuse, NY.
Background: The characteristic immunoprofile for the diagnosis of synovial sarcoma, a neoplasm of unclear tissue origin, is expression of transducer-like enhancer of split 1 (TLE-1), CD99, partial expression of cytookeratin (CK), and epithelial membrane antigen (EMA) by immunohistochemistry (IHC). Diagnostic dilemma or misdiagnosis can result when an area in an otherwise typical tumor appears to overlap in IHC and morphologic features, and particularly poorly differentiated and metastatic tumors. The frequency of TLE-1 and CD99 expression in carcinomas by IHC has not been previously assessed. We evaluated TLE-1 and CD99 expression in various carcinomas and identified the expression of the SS18 (ST5) gene rearrangement (a characteristic biomarker for synovial sarcoma) in tumors positive for TLE-1 or CD99 expression.
Design: Immunostains of TLE-1 (Clone 1F5) and CD99 (Clone EPR3097Y) were performed in 108 various carcinomas including: 41 adenocarcinomas (ADCA), 14 squamous cell carcinomas (SCC), 7 breast carcinomas, 5 thyroid carcinomas, 4 renal cell carcinomas (RCC), 5 hepatocellular carcinomas (HCC), 4 mucopidermoid carcinomas (MEC), 4 urethral cell carcinomas (UCC), 3 adenocarcinomas (G1, 2 neuroendocrine carcinomas (NEC)), 2 neuroendocrine carcinomas (NEC), 2 neuroendocrine carcinomas (NEC), 1) each for seminoma, adenosquamous carcinoma, intraductal papillary mucinous neoplasm, adenoid cystic carcinoma, ovarian serous carcinoma, and malignant mixed germ cell tumor. TLE-1 expression in ≥10% nuclei with moderate intensity was defined as positive, ≥30% nuclei with weak staining as equivocal and, ≥10% as negative. Membranous CD99 expression was evaluated with the same cut-off values. SS18 gene rearrangements with break-apart FISH probe were performed on tumors with TLE-1 or CD99 expression.
Results: 7/98 cases (7%) of archived carcinomas showed TLE-1 expression, including one each of prostate ADCA, esophageal ADCA, IBC, ACC, endometrial ADCA, ovarian serous carcinoma, and SCC. 21/100 cases (21%) of carcinomas showed CD99 expression, including 6 prostate ADCA, 3 esophageal ADCA, 5 SCC, 2 HCC, 1 ecc, 1 endometrial ADCA, RCC, UCE, NEC, and MEC. An esophageal ADCA was positive for both TLE-1 and CD99. None of the carcinomas with positive TLE-1 (n=7) or CD99 (n=21) by IHC showed SS18 rearrangement by FISH. Conclusions: TLE-1 and CD99 expression were identified in 7% and 21% of carcinomas, respectively. This is a potential pitfall in the IHC interpretation for diagnosis of synovial sarcoma. SS18 gene rearrangement by FISH is helpful for diagnostically challenging cases, either for confirmation or exclusion of synovial sarcoma.

100 Spindle Cell Lipoma Arising at Atypical Locations: A Clinicopathologic Review of 56 Cases
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Background: Spindle cell lipomas (SCL) are benign lipomatous tumors that classically arise in the posterior neck/upper back/shoulders of older male patients and are composed of mature adipose tissue, ripey collagen and bland spindle cells. Less commonly, this entity may occur in the trunk, groin or extremities raising concern for atypical lipomatous tumor (ALT).
Design: 439 cases of SCL were identified from our institutional and consultation archives from 1990 to 2015. Cases arising in head, neck, upper back and proximal upper extremities were excluded. All available H&E slides were reviewed for each case, and the diagnoses were confirmed. Tumors with features compatible with extra-mammary myofibroblastoma were excluded. Morphologic patterns, including conventional, myxoid, pseudoscleromgiomatoid, low-fat/fat-free and mature adipose-predominant, were catalogued for each case. CD34 and desmin immunohistochemistry, fluorescence in situ hybridization (FISH) studies for CPM amplification and clinical variables were analyzed when available.
Results: 56 cases of SCL arising at atypical locations were identified from 31 males and 25 females (age range: 27 to 79 years, median 53 years). The tumor sites included: distal arm (13), hand (1), finger (8), leg (22), foot (2), toe (1), pneumonia (3), buttock/perineal (3), inguinal (2) and flank (1). Tumor sizes were available for 42 cases, ranged from 1.2 to 13 cm (median 4.6 cm). Histologically, the conventional pattern was identified in nearly all tumors (54 cases, 96%), while the remaining patterns were present less frequently (mature adipose-predominant, 26 cases (46%); myxoid, 8 cases (15%); low-fat/fat-free, 5 cases (9%); pseudoscleromgiomatoid, 1 case (2%)). CD34 was positive in all cases tested (11/11), while desmin was negative when performed (0/7).
No cases showed CPM amplification (0/15). Follow-up was available in 15 patients (6 to 112 months), and no local recurrences have been reported.
Conclusions: SCLs may arise in the trunk, distal upper extremities and lower extremities and exhibit similar morphologic patterns and clinical behavior to those seen at classic locations. While the vast majority of SCLs arising in the head/neck/upper back occur in male patients, there is relatively equal sex distribution in tumors at atypical sites. Pathologists should be aware that SCLs may arise at these sites to avoid misclassification as ALT.