Molecular Diagnosis of Soft Tissue Tumors: Avoid Pitfalls

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Overview

I. When should we rely on the help of molecular testing?

II. Specificity, Prevalence, and Prognostic Implications of Molecular Abnormalities.

III. ‘Gold Standard’ in surgical pathology of soft tissue tumors

I. When should we rely on the help of molecular testing?

– difficult distinctions between a benign and malignant diagnosis

– unusual morphologic features

– unusual clinical presentations or unexpected immunohistochemical results

– refined classification may directly impact on clinical management of the patient

– confirm diagnosis in conflicting second opinions

Benign vs Malignant STT

– Perineurioma versus Low Grade Fibromyxoid Sarcoma

– SFT/HPC versus Synovial Sarcoma (SS)

– Angiomatoid Fibrous Histiocytoma vs sarcoma

– Lipoblastoma vs myxoid liposarcoma in children

Sarcomas with unusual morphologic appearance

– Desmoplastic Round Cell Tumor (DRCT) with predominantly rosettes or tubular structure formation

– Small cell GIST in children

STT with typical morphology but unusual clinical presentation

– Ewing Sarcoma in superficial location

– Ewing Sarcoma in older individuals (>40 years)

– Ewing Sarcoma in visceral location
STT associated with an unusual immunoprofile

- Ewing Sarcoma (ES) with strong Keratin expression
- Synovial sarcoma with S100 protein positivity

Refined classification may directly impact on clinical management

- GIST versus sarcoma, NOS
- Ewing Sarcoma vs Poorly differentiated Synovial Sarcoma
- ARMS vs ERMS

Refinement in diagnosis impacts on outcome or clinical follow-up

- Liposarcoma with extensive myxoid changes in the retroperitoneum
- HG sarcomas of the extremities: dedifferentiated liposarcoma vs MFH

Confirming diagnosis when in disagreement with other expert review

- Disprove a diagnosis of Synovial Sarcoma in an MPNST
- Confirm a certain sarcoma subtype (ARMS vs ERMS)

Applying Molecular Diagnosis in Other Clinical Scenarios

- Establish a primary diagnosis of sarcoma versus metastatic disease
  - Primary GI clear cell sarcoma vs metastatic melanoma to the gut

Applying Molecular Diagnosis

- In cases with discordant results between morphology and cytogenetic results:
  - Pediatric tumor with an EWSR1 gene rearrangement does not always equal an Ewing sarcoma
    - Angiomatoid Fibrous Histiocytoma (AFH)
    - Myoepithelioma (ME)
II. Specificity, Prevalence, and Prognostic Implications of Molecular Abnormalities

• Fusion transcripts have been proved not only to be highly specific molecular diagnostic markers, but their prevalence in most sarcomas is such that they come to define these entities.

• The molecular heterogeneity of fusion transcripts has been suggested to have a prognostic role in certain sarcoma types (Ewing sarcoma, ARMS).

III. ‘Gold Standard’ in surgical pathology of soft tissue tumors

• In most cases molecular results should be used as validation of the morphologic differential diagnosis, corroborated with immunohistochemical findings and clinical information, rather than a challenge to the supremacy of histopathology.

Caveats of wide-availability of certain genetic tests

– are now being applied on archival material, in Academic Institutions or Private labs, lacking appropriate sarcoma pathology expert review:

– FISH test is looking at one partner at the time
  • Promiscuity of certain translocation partners: EWSR1 gene family of tumors (ES, DRCT, EvMyxCS, MLS, CCS, GI CCS, AFH, ...ME,...?).
  – Diagnostic pitfalls triggered by misinterpretations of gene rearrangements results, with automatic classification in major translocation-positive sarcomas
Ultrastructural Diagnosis of Soft Tissue Tumors: Avoiding Pitfalls

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Introduction

The use of immunohistochemistry and, more recently, molecular methods has seen a sharp reduction of the role of electron microscopy in the diagnosis of soft tissue sarcomas (1,2). Ultrastructural analysis, however, does continue to play a useful role in selected cases. The focus in this presentation is on adult soft tissue tumors; Dr. John Hicks will follow with a presentation on avoiding pitfalls in the ultrastructural diagnosis of pediatric soft tissue tumors.

Before moving to the issue of pitfalls, consideration should be given to the current role of electron microscopy in diagnosis of soft tissue tumors. One of the most obvious types of cases of adult soft tissue tumors for which electron microscopy can prove useful is in the definitive classification of malignant peripheral nerve sheath tumors. Only 50 to 70 percent of these cases will demonstrate any S-100 staining, yet the ultrastructure is generally diagnostic (1). Without ultrastructural study, many of these cases fall in an unclassified status or are classified as malignant fibrous histiocytomas. Another obvious example is alveolar soft part sarcoma cases not identified by light microscopy.

Other instances include cases where the sample size is too small to permit adequate immunohistochemical study, including fine needle aspirates (3). Additional examples are poorly differentiated lesions with unclear immunohistochemical results in which the goal is to distinguish a sarcoma from a sarcomatoid carcinoma (3,4).

Many other applications for electron microscopy in the evaluation of adult sarcomas arise, but generally in a small subset of any given entity or in some rare variants (1,4). These include:

1. Epithelioid fibrosarcoma (which may be EMA positive) and some typical cases of fibrosarcoma (which have no specific markers).
2. Low grade myofibroblastic sarcoma (for definitive distinction from a smooth muscle tumor).
3. Smooth muscle tumors with negative or very focal immunohistochemical staining.
4. Very rare cases of liposarcoma, particularly pleomorphic liposarcoma, when the diagnosis is in doubt (e.g., distinguishing the epithelioid variant of pleomorphic liposarcoma from adrenal cortical carcinoma (5)).

5. Angiosarcomas lacking definitive immunohistochemical findings.

6. Translocation-negative monophasic synovial sarcomas.

7. Epithelioid sarcoma vs. epithelioid angiosarcoma with a CD34+, CD31-, cytokeratin+ immunophenotype.

8. Malignant perineurial cell tumors (rare).

Pitfalls

Within this selected set of cases for which electron microscopy remains highly useful, however, certain pitfalls do arise. And as electron microscopy is less frequently used for tumor analysis than in the past, individuals performing the electron microscopy are more and more likely to have primary ultrastructural expertise in an area other than tumors, and awareness of these relatively few pitfalls may prove helpful.

Malignant Peripheral Nerve Sheath Tumor vs. Monophasic Synovial Sarcoma

Again, one of the most common applications for electron microscopy in the evaluation of adult soft tissue tumors may be in definitive classification of malignant peripheral nerve sheath tumors. These tumors may be recognized ultrastructurally by interdigitating cell processes and discontinuous external lamina. While the pattern is fairly specific, it can be mimicked. Monophasic synovial sarcoma classically features bipolar tapering processes, but cases can demonstrate similar interdigitating cell processes. Further, while monophasic synovial sarcoma generally lacks a true external lamina, a flocculent amorphous substance is characteristically identified between cells. As this material presses against the plasma membrane, it can closely mimic a discontinuous external lamina. In the absence of rudimentary epithelial elements – or failing to look for them given what appears to be a classical pattern of a malignant peripheral nerve sheath tumor – one may conclusively classify the lesion as a malignant peripheral nerve sheath tumor ultrastructurally and fail to explore the classic translocation. A key to avoiding this pitfall is the extent of the intercellular amorphous material, though this is a subjective measure. Fortunately, most cases of monophasic synovial sarcoma will not manifest the interdigitating pattern of cell processes.
**Crystal-Deficient Alveolar Soft Part Sarcoma**

Another ultrastructural pattern which is usually entirely straightforward is that of alveolar soft part sarcoma. Indeed, these tumors sometimes exhibit intracytoplasmic, PAS-positive crystals which are several microns in maximum dimension and readily seen by light microscopy; such cases require no ultrastructural study. Other cases represent a classic application of electron microscopy for tumor analysis, as large rhomboidal crystals with a 10 nm periodicity permit a specific diagnosis. The pitfall is that, despite this classical description, some cases of alveolar soft part sarcoma lack this feature (6). In these, the key feature instead is the presence of numerous secretory granules (as well as prominent rough endoplasmic reticulum and frequent mitochondria). With luck, one may still identify the typical early crystallization within some of the granules, but in some cases, even this may not be found (7).

**Smooth Muscle Tumors with Few Filaments**

As noted in the list of applications of electron microscopy for soft tissue tumors, occasionally a diagnosis of a smooth muscle tumor may be made ultrastructurally in a case in which immunochemistry for actin was negative or too limited to be conclusive. The pitfall in this case is to avoid falsely excluding a smooth muscle tumor ultrastructurally. While some cases will have numerous filaments and dense bodies as well as extensive external lamina and pinocytotic vesicles, such cases will tend to give reliable immunohistochemical results. For the preselected cases which will present for electron microscopy, only scanty filaments may be present, usually peripherally, especially if the lesion is epithelioid (8,9). The presence of numerous mitochondria reinforces the concept of smooth muscle differentiation and should prompt further search for filaments. And in contrast to myofibroblasts, rough endoplasmic reticulum should be scanty.

**Myofibroblasts**

By immunohistochemistry, myofibroblasts cannot be reliably distinguished from smooth muscle cells. Electron microscopy, then, can be highly valuable for definitive classification of low grade myofibrosarcoma. Identifying tumor cells with myofibroblastic differentiation, however, is not specific for a diagnosis of myofibrosarcoma; myofibroblastic differentiation is also seen in tumor cells of fibromatosis, fibroma of tendon sheath, myxofibrosarcoma, malignant fibrous histiocytoma, and other tumors (7). Identifying myofibroblasts in a tumor, though, does not indicate that it is one of these lesions. Myofibroblasts are very common reactive cells in a large variety of tumors, both soft tissue and epithelial (10). Further, the nuclei of reactive myofibroblasts are not readily distinguished in many cases from neoplastic ones. Care must be taken to establish that the cells are representative of the lesion.
Lipid

One might think that ultrastructure would never have a role in the diagnosis of liposarcoma, and indeed the use of electron microscopy in the diagnosis of liposarcoma is quite rare. Some cases of pleomorphic liposarcoma, however, can express epithelial or myogenic markers, and in such cases, electron microscopy may prove useful for definitive diagnosis (4,5). It may also prove useful in some cases in which vacuolated cells are seen, but no definite lipoblasts are identified (4). In this latter example, unless the vacuoles are explained, negative results are meaningless given the sampling issues. And finding lipid droplets in tumor cells is a highly nonspecific finding, as lipid can accumulate in tumor cells damaged by intrinsic anoxia or therapy, and neoplastic cells infiltrating adipose tissue may contain lipid from phagocytosis of the fat (7).

Desmosomes

Very infrequently, electron microscopy is undertaken simply to determine whether an anaplastic lesion is a sarcoma or a carcinoma, such as when immunohistochemistry is unsatisfactory or inconclusive or insufficient tissue is present for adequate analysis. Such studies are often frustrating, as one might expect. A positive finding that generally indicates carcinoma rather than sarcoma is the detection of desmosomes. One should bear in mind, however, that desmosomes may be found in synovial sarcoma and epithelioid sarcoma (7).

Conclusion

While electron microscopy is much less commonly employed in the diagnosis of adult soft tissue tumors than previously, in a subset of these lesions, ultrastructural study remains highly beneficial for diagnosis. Relatively few pitfalls exist. Knowledge of the current applications of electron microscopy for the diagnosis of these lesions and of the pitfalls in those examinations permits optimal utilization of the electron microscopy laboratory in contributing to the care of these patients.


Ultrastructure as an Important Component in Diagnosis of Pediatric Soft Tissue Tumors:
Avoiding Pitfalls

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Triaging of Tissue
Small round cell tumors of childhood have undergone extensive analysis for morphologic, cytogenetic, molecular, biochemical and biopathologic factors that influence response to oncologic management and overall survival. Many different protocols have been developed and continue to be instigated based upon clinical, pathologic and molecular study of specific tumor types. The pathologist is intimately involved in assuring that tissue is properly submitted for the variety of pediatric tumor protocols. A general schema for triaging tissue from pediatric tumors is presented (see Table). Paramount to evaluation of these tumors is providing adequate tissue for intraoperative interpretation and final diagnosis. The pathologist may elect to perform a frozen section and/or cytologic imprints in order to formulate an opinion. Once the pathologist determines that adequate tissue has been obtained for tumor diagnosis, assessment of the amount of residual tissue available for cytogenetics, molecular analysis, and biologic study is necessary. If tissue is inadequate, the surgeon may be able to provide additional tissue to meet the needs for protocol studies. In the event that the surgeon is not fully able to obtain adequate tissue, the pathologist will need to determine the priority of various components of the tumor protocol and submit the tissue accordingly. In general, submission of tissue for cytogenetics, and retention of the frozen section tissue block at -70°C for molecular diagnostic, RT-PCR, biochemical and microarray gene product analyses will allow for assessment of factors important for oncologic management in solid and hematolymphoid tumors. The preparation of cytologic imprints from fresh tissue can be performed prior to submitting the tissue for other protocol studies. Cytologic imprints allow for fluorescent or chromogen in situ hybridization (FISH/CISH) evaluation of mutated genes, tumor-defining translocations and other cytogenetic abnormalities. It is also possible to identify amplification or copy numbers of biologically important factors, such as MYC-N amplification that is associated with worse prognosis and decreased survival in neuroblastoma. With lymphomas and leukemias, determination of cell surface markers by flow cytometry is also critical in determining the diagnosis.

When a specific pediatric tumor type is suspected based upon clinical and imaging studies, the oncologist and the tumor protocol coordinator should be able to provide the pathologist with the tissue protocol prior to biopsy or resection of the tumor mass. The vast majority of children and adolescents with tumors are eligible for and will be registered on Children's Oncology Group (COG) treatment protocols. Consultation with a pediatric pathologist or hematologist/oncologist at a COG-affiliated institution will allow for proper triaging of tissue for oncologic management and for placement on an appropriate protocol based upon the biopathology of the tumor.

Oftentimes, soft tissue masses and lymph nodes will not yield tumorous tissue. Instead, inflammatory or infectious processes will be encountered. Fresh tissue for microbiologic and viral cultures should therefore be obtained to allow for identification of an infectious agent. Bacterial, acid-fast bacilli and viral organisms also may be detected by RT-PCR, molecular
microbiology and/or virology techniques as well, and the frozen tissue will additionally enable immunofluorescence detection of autoimmune or immune-mediated diseases.

**Infantile Myofibromatosis (myofibromatosis, myofibroma)**

Although initially described some 5 decades ago as congenital fibrosarcoma, this entity was quickly reclassified as congenital generalized fibromatosis in 1954 when it was recognized that this spindle cell tumor lacked malignant potential. Review of a large number of cases resulted in recognition of the myofibroblastic nature of the tumor and the tumor was renamed infantile myofibromatosis. There are 3 forms: solitary (most common >50%), multicentric (less common, 33%) and multicentric (uncommon, <15%) with visceral involvement. The solitary form is usually cutaneous with dermal involvement and extension into the underlying subcutis, muscle and even bone. Several soft tissue sites may be involved alone (multicentric) or concomitantly with lung, cardiac, gastrointestinal or even central nervous system involvement (multicentric with visceral involvement). Solitary or multicentric bone lesions may also be present as the sole type of lesions. The prognosis for solitary and multicentric forms is excellent; whereas, >75% of infants with visceral involvement die of disease. Many of the lesions without visceral involvement stabilize and may undergo spontaneous regression. Some of the solitary lesions may become locally aggressive with unremitting gradual destruction of normal tissues. Particularly aggressive tumors in non-resectable sites may require chemotherapy, similar to that for infantile fibrosarcoma in order to allow for resection or alleviate dysfunction. The head and neck followed by the extremities and trunk are the most common sites of involvement.

The histopathology of myofibromas is characterized as a nodular or multinodular proliferation with a zoning phenomenon, characterized by peripheral spindle-shaped cells organized into fascicles. These tend to merge and blend with centrally placed sheets of less differentiated ovoid to polygonal shaped cells. A prominent pericytoma architecture may be seen throughout the tumor, but more prominently within the center of the tumor. The recognition of this pattern has resulted in inclusion of the tumor previously considered to be infantile hemangiopericytoma into the infantile myofibromatosis category. This is appropriate because these tumors lack the cytogenetics features and other features of true adult hemangiopericytoma (solitary fibrous tumor, as reclassified by the WHO). Myofibromas with this pattern are often referred to as myofibromas with a hemangiopericytoma-like pattern. Myofibromas may have a relatively high mitotic rate without atypical mitotic figures, areas of necrosis and calcifications, stromal hyalinization, nuclear atypia and even subendothelial “intravascular” tumor growth. These histopathologic features have no bearing on the clinical outcome of myofibromas. Immunocytochemical staining of the tumor cells reveals vimentin and alpha-smooth muscle actin reactivity, with lack of immunoreactivity with S100 protein, epithelial membrane antigen, keratin and desmin. Unlike desmoid tumors (deep fibromatoses) that have a somatic beta-catenin or APC gene mutations that lead to beta-catenin nuclear accumulation, beta-catenin is expressed in the cytoplasm and not in the nucleus with myofibromas/myofibromatosis. In fact, beta-catenin is not expressed in a nuclear pattern with low-grade fibromyxoid sarcoma, leiomyosarcoma, myofibrosarcoma, sclerosing epithelioid fibrosarcoma, low-grade fibrosarcoma, classic fibrosarcoma, dermatofibrosarcoma protuberans, myxoinflammatory fibrosarcoma, nodular fasciitis or scars.

Ultrastructural examination of myofibromas shows myofibroblastic differentiation with prominent dilated rough endoplasmic reticulum, longitudinal filaments with dense bodies, and focal basal lamina. Fibronexus structures may be found in a limited number of cases. It should be emphasized that intercellular junctions, pinocytotic junctions and basal lamina are found in myofibroblastic tumors; however, these are found much more commonly in rhabdomyosarcomas. This may give the false sense of a benign fibroblastic lesion when striated muscle differentiation (well-formed external lamina, monoparticulate glycogen, myofilaments
and Z-band material) is expressed ultrastructurally in infrequent to rare tumor cells. Immunocytochemical studies should be performed in those cases that do not provide convincing evidence of myofibroblastic differentiation (peripheral cytoplasmic filament arrays).

Cytogenetic analyses of myofibromas have shown nonspecific findings with chromosome 8 abnormalities. A small number of reports of familial myofibromatosis have implicated an autosomal dominant inheritance pattern. It must be emphasized that the vast majority of myofibromas are sporadic, isolated occurrences. Most importantly, these tumors lack the tumor-defining translocation (ETV6-NTRK3) found with infantile fibrosarcoma and cellular mesoblastic nephroma. In particularly troublesome cases, RT-PCR or FISH for the ETV6-NTRK3 translocation should be performed to eliminate infantile fibrosarcoma as a consideration.

**Congenital Infantile Fibrosarcoma (congenital fibrosarcoma, infantile fibrosarcoma)**

This malignant fibroblastic tumor presents during the first year of life, or even at birth, in the majority of cases and may be detected *in utero* in some instances. The trunk and extremities are most often involved. Unlike adult fibrosarcoma, this tumor usually involves the distal portions of the extremities. The infant presents with an asymptomatic, rapidly growing, bulky tumor that is located in the deep soft tissues. These tumors tend to be locally invasive and metastasize infrequently. Conservative resection with negative surgical margins is the key to avoiding recurrence. Outcome is dependent upon the site of the tumor with low survival rates for retroperitoneal tumors and high survival rates for extremity tumors. With large nonresectable tumors, chemotherapy based upon rhabdomyosarcoma protocols has proven beneficial in reducing tumor size and allowing for adequate resection. Many pediatric oncologists now consider chemotherapy for congenital infantile fibrosarcoma (CIF) in order to reduce tumor volume and limit the need for more radical surgery, especially when amputation may be a concern prior to initial evaluation.

Gross examination reveals an infiltrative fleshy tumor that lacks a well-defined border. The cut surface of the tumor tends to be lobulated with a myxoid to mucinous character with areas of necrosis, cystic degeneration and hemorrhage. The tumor is a highly cellular neoplasm with a herring-bone pattern. The cells are spindled in outline, but have a high nuclear to cytoplasm ratio. There is considerable nuclear hyperchromasia, tumor necrosis and frequent mitoses scattered throughout the tumor. The tumor cells are closely packed and overlap one another. Focal hemangiopericytoma-like areas with increased vascularity may be seen. These tumors typically are diffusely positive for vimentin and focally reactive for actin. Electron microscopy provides evidence for fibroblastic differentiation. The ultrastructural features of lack of to extremely rare extracellular banded collagen, rare to absent basal lamina, branching and markedly dilated rough endoplasmic reticulum, irregular nuclear membranes, and extracellular fibrillogranular material are helpful in separating this malignant fibroblastic tumor from myofibromas. These features are also more characteristic of origin from a primitive mesenchymal spindle cell, representing a precursor cell in the fibroblastic/myofibroblastic cell line. Electron microscopy plays a critical role in distinguishing this malignant tumor (CIF) from spindle cell rhabdomyosarcoma, infantile rhabdomyofibrosarcoma, undifferentiated sarcoma and benign fibroblastic/myofibroblastic neoplasms. Ultrastructural examination is particularly important with the approximately 20 to 30% of infantile fibrosarcomas that immunoreact with desmin and/or muscle specific actin, and may result in confusion with a spindle cell rhabdomyosarcoma. An extensive search for striated muscle differentiation (well-formed external lamina, monoparticulate glycogen, myofilaments and Z-band material) should be undertaken with a spindle cell tumor possessing a predominance of banded collagen and absence of extracellular matrix fibrillogranular material. In addition, the herring-bone pattern, nuclear hyperchromasia, necrosis and cystic degeneration distinguish this tumor from a benign myofibromatous tumor. An unusual childhood tumor that may be confused with infantile
fibrosarcoma is the infantile rhabdomyofibrosarcoma (IRMFS, see below). The identification of rhabdomyoblasts by immunocytochemistry, or more likely by electron microscopy, help to define the high-grade infantile rhabdomyofibrosarcoma. Congenital infantile fibrosarcoma shares a tumor-defining translocation (ETV6-NTRK3) with cellular mesoblastic nephroma. In addition, it may have other cytogenetic abnormalities, such as trisomy of certain chromosomes (trisomy 8, 11, 17, 20).

The prognosis for CIF is quite favorable with complete wide local excision usually being curative. If the mass is not resectable, chemotherapy with vincristine and adriamycin are employed to reduce tumor size and allow for wide local excision. This is accomplished following chemotherapy in the majority of initially nonresectable tumors. The overall survival at 5 years is at least 80%.

**Infantile Rhabdomyofibrosarcoma**

Infantile rhabdomyofibrosarcoma (IRMFS) is a rare, under-recognized entity that was first described in 1995 with only 11 cases reported in the literature to date. The tumors occur early in life with an age range from 3 days to 4 years, with the majority of tumors being present by age 18 months of age (about 80%). A variety of sites have been involved and include the distal extremities (3), trunk (1), buttocks (1), forearm (1), pelvis and prostate (1), intrathoracic/extrapleural (1), and head and neck (1). Histopathologically, the tumor appears similar to CIF, being composed predominantly of spindled fibrosarcomatous tumor cells, with only rare ovoid to spindled rhabdomyoblastic cells. These rhabdomyoblastic cells may be "cryptic", because these tumor cells tend to be hidden among the spindled fibrosarcomatous cells that lack overt rhabdomyoblastic differentiation. The rhabdomyoblastic tumor cells lack cross-striations. Immunocytochemistry for desmin and myogenin, as well as other myogenic markers, may help to differentiate the infrequent to rare rhabdomyoblastic tumor cells from the more abundant fibroblastic tumor cells. Electron microscopy is also of great utility in discovering rhabdomyoblastic tumor cells that characteristically possess myofilaments, Z-band material and glycocalyx coatings. In addition, the dense fibrillogranular material within the stroma that is characteristic for CIF is also present with IRMFS. The stroma also contains fine fibrillar material and infrequent collagen fibers similar to CIF. IRMFS differs from typical spindle cell embryonal rhabdomyosarcoma in anatomical distribution, degree of myogenic differentiation and in prognosis. More recently, it has been shown in a limited number of IRMFS cases (n=3) that the CIF-associated ETV6/NTK3 translocation may be detected by cytogenetics or FISH chromosomal rearrangement. This supports IRMFS being a variant of CIF. As noted previously, monosomy for chromosomes 19 & 20 are also common cytogenetic findings.

Of considerable importance in differentiating CIF from IRMFS is the aggressive nature and metastatic ability of IRMFS. In fact, IRMFS is considered to be a high-grade sarcoma. In contrast, CIF has virtually no metastatic potential, and deaths are usually associated with local progression of disease. With IRMFS, metastatic tumor to the lungs, liver and mediastinum have been reported in about 50% of cases. Local recurrence has been noted in about 60% of IRMFS cases. Death associated with metastatic disease has been reported in about 40% of IRMFS cases, with only limited follow-up available. Because of the aggressive nature of IRMFS, wide local excision and chemotherapy are recommended. Currently, rhabdomyosarcoma chemotherapy is recommended to avoid metastatic disease and reduce local tumor burden. If widely local excision can not be performed or if there are positive surgical margins, radiation therapy in addition to chemotherapy will be performed.

**Pericytoma with t(7;12)**

A distinct pericytic tumor with a recurrent novel translocation involving the short arm of chromosome 7 and the long arm of chromosome 12 has recently been recognized. This under-recognized tumor may be mistaken for a myofibroma with a prominent hemangiopericytoma-like
pattern or a true adult hemangiopericytoma (solitary fibrous tumor, as reclassified by the WHO). These tumors have involved a spectrum of ages (11 to 65 years) and sites (tongue, stomach, lower leg). These tumors were characterized by multi lobulation and infiltrative growth of spindle-shaped tumor cells arranged around thin-walled small vessels. The tumor cells were subendothelial in location. Cytologic atypia and pleomorphism were lacking. Mitoses were infrequent. Immunocytochemistry revealed smooth muscle actin, laminin, and type IV collagen. The tumor cells were not immunoreactive with S100 protein, keratin, desmin or CD34. Ultrastructural features supported a pericytic origin with incomplete basal lamina, subplasmalemmal thickenings, and thin filaments with focal dense bodies along the periphery of the cytoplasm. Considering the overall features, this tumor may become classified within the myopericytoma category, according to the WHO soft tissue guidelines.

A novel translocation associated with this tumor involves the beta-actin gene (ACTB) and GLI oncogene [t(7;12)(p21-22;q13-15)]. This fusion gene may result in overexpression of GLI by the inclusion of a promoter region from ACTB. GLI is essential in the sonic hedgehog signaling pathway which participates in cell cycle regulation, cell adhesion, apoptosis, signal transduction and cell proliferation.

These pericytic tumors are limited in number; however 3 of the 5 tumors were resected with negative margins and have not recurred. Two of the tongue tumors required chemotherapy in order to decrease tumor size and to allow for gross total resection. No recurrences or metastatic disease were reported over a mean follow-up period of 24 months.

More recently, inhibition of GLI-mediated transcription and tumor growth by small molecule antagonists has been reported. These antagonists are targeting the Smo-tened proto-oncogene which affects the SUFU gene in the Hedgehog signaling pathway and the downstream GLI pathway implicated in pericytoma with t(7;12); medulloblastoma, prostate cancer, rhabdomyosarcoma, lung cancer and pancreatic adenocarcinoma. The inhibitors (Gli-antagonists - GANT 58, GANT 61) of the GLI pathway act in the nucleus to block GLI function and to interfere with GLI-DNA binding. GANT 58 and GANT 61 have been shown to inhibit cell proliferation and block cell growth in \textit{in vivo} xenograft models using human tumor cell lines harboring downstream activation of the Hedgehog pathway, including the GLI pathway. This discovery holds promise for treatment of tumors with alterations in the GLI pathway, as occurs in pericytoma with t(7;12).

\textbf{Biphenotypic Sarcoma}

Biphenotypic sarcomas are a subset of pediatric sarcomas with both myogenic and neuroectodermal differentiation. The number of reported cases is quite limited (<20). The gender ratio is equal. The age range is from 5 months to 25 years, with most being in the adolescent age group. The sites of involvement include intrabony sites (femur) and deep soft tissue sites (abdomen, chest wall, thigh, upper arm, retroperitoneum, hand). The initial diagnosis rendered in the majority of cases was rhabdomyosarcoma. Long-term followup is not available on most of the cases reported in the literature; however, these tumors tend to quite aggressive and metastasize readily.

The tumor cells tend to be polygonal to stellate to spindled. The nuclei are irregular and somewhat vesicular, and lack prominent nucleoli. The indistinct cytoplasm tends to be scant, amphophilic to lightly eosinophilic. There is a paucity of stroma, but with a fine vascular network. There are occasional apoptotic cells, but usually minimal necrosis. Mitotic activity is usually brisk (>10/10 hpf). The immunophenotype is both myogenic (desmin, actin, myogenin, MYF5, MRF4) and neuroectodermal (NSE, NFP, PGP 9.5, chromogranin, CD99, acetylcholinesterase, S100 protein). Both vimentin and keratin are expressed in some tumors. The tumors are negative for CD45, NB84 (neuroblastoma) and MyoD1. The ultrastructure demonstrates myofilaments varying from rudimentary myofilaments to actin-like filaments to z-bands. Also,
there are tumor cells with neural features, such as neuritic processes, dense core neurosecretory granules and synaptic-like junctions.

Most of these tumors express Ewing sarcoma translocations [EWS-FLI1: t(11;22); EWS-ERG: t(21;22)] with both oncoprotein and transcription factor expression associated with these translocations. As noted previously, myogenic regulatory gene expression is detected using immunocytochemistry for desmin, actin, myogenin, and actin-like intermediate filaments, as well as with electron microscopic identification of myogenic features. Neuroectodermal gene expression is identified with immunocytochemistry for neuroectodermal markers (NSE, chromogranin, synaptophysin, CD99) and with electron microscopic identification of glycogen pools and dense core neurosecretory granules. Both myogenic and neuroectodermal features are identified within tumor cells from the same tumor. RT-PCR and cytogenetic studies have demonstrated EWS-FLI1 and EWS-ERG translocations, and both EWS-FLI1 and PAX3-FOXO1A translocations in a single case. FISH studies can identify translocation of EWS and, in a rare case, translocation of FOXO1A. Additional molecular studies have shown expression of myogenic regulatory genes for myogenin and MYF5, as well as neurofilament genes (NF-H, NF-L) and neural genes (CAT, CGA, NSE). It is believed that biphenotypic sarcoma is derived from pluripotent embryonic neural crest tissue ectomesenchyme which may be widely dispersed in the body. Of interest is the fact that MyoD1 is not expressed in biphenotypic sarcoma. This is expected because EWS-FLI1 and EWS-ERG translocations inhibit MyoD1 expression in a similar fashion as Ras, c-Fors and C-Jun. This allows for neuroectodermal differentiation. Some investigators believe that biphenotypic sarcoma may represent a form of malignant ectomesenchymoma (MEM). MEM is neural crest derived and has both neural and malignant mesenchymal components, most often rhabdomyosarcoma. However, neurofibrosarcomatous elements with ganglion cells have not been reported to date with biphenotypic sarcoma. MEM is considered by many to represent a member of the Ewing family of tumors. Several MEMs have been reported to have Ewing sarcoma translocations.

An accurate diagnosis is important with biphenotypic sarcoma. This is because these tumors are treated on Ewing sarcoma protocols, rather than rhabdomyosarcoma protocols. These tumors tend to be highly aggressive and require intensive high-risk Ewing sarcoma-based therapy. As noted previously, the majority of tumors were initially thought to be rhabdomyosarcoma at the original institutions where biopsies were performed. It should be noted that some of these tumors have been reported as small round cell tumors with biphenotypic neuroectodermal and myogenic differentiation associated with a Ewing's translocation. In one case, the diagnosis provided indicated a biphenotypic sarcoma with characteristics of both Ewing sarcoma and desmoplastic small round cell tumor (DSRCT). This particular tumor had an EWS-FLI1 translocation, but lacked the criteria necessary to classify the tumor as a DSRCT and lacked the EWS-WT1 translocation associated with the vast majority of DSRCT. This emphasizes the need for knowledge of this tumor, and applying the appropriate immunocytochemical, ultrastructural and molecular diagnostic studies. It has been estimated that as many as 10% of tumors diagnosed as rhabdomyosarcomas may be biphenotypic sarcoma.

**Sclerosing Epithelioid Fibrosarcoma**

Sclerosing epithelioid fibrosarcoma (SEF) is a distinctive variant of fibrosarcoma which is quite uncommon. It occurs in a wide age range, from adolescent to late adulthood (14-55 years) with an equal gender ratio. Over the past 2 decades, there have been about 90 cases reported. The tumors may be present for months to years. Only on-third of patients experience pain and some have extremity weakness. With chest masses, shortness of breath and persistent cough may be present. The sites of involvement are usually the lower extremities, limb girdle, trunk, upper extremities and head and neck region. The tumors tend to be deep
seated and impinge upon bone, although they rarely invade bone. Because of the impingement on bone, the clinician and pathologist may be mislead in thinking that the tumor is bone-derived. Tumor size is quite variable, being from 2 to 22 cm with a median between 7 to 10 cm reported. The tumors tend to be well-circumscribed and lobulated to multinodular. The cut surface is firm and white with some myxoid, cystic or calcified areas. Necrosis is uncommon. The tumor is composed of epithelioid cells arranged in nests, cords, strands or acini with scant amphophilic to eosinophilic cytoplasm. There tends to be abundant collagen that is variably arranged into bands, delicate lace-like fibrosis, and hyalinized fibrosis. There may be areas that resembles low grade fibrosarcoma, low grade fibromyxoid sarcoma, myxoma, or myxofibrosarcoma. The tumor may also be confused with poorly differentiated carcinoma or sclerosing lymphoma. Metaplastic bone may be present, as well myxoid cystic regions. A hemangiopericytoma-like vasculature may be seen. Vascular invasion may be seen at the periphery of the tumor. Mitotic activity is quite variable.

Immunocytochemistry shows typical mesenchymal markers, such as vimentin. There may be focal weak EMA and S100 protein reactivity, as well as rarely cytokeratin positivity. MDM2 and p53 are overexpressed in these tumors. Electron microscopy demonstrates the fibroblastic nature of the tumor with parallel arrays of rough endoplasmic reticulum with granular material filing the cisternae. There may be prominent networks of intermediate filaments that form perinuclear whorls. No basal lamina material is seen.

Molecular and cytogenetic studies have identified amplification of 12q13 and 12q15 (HMGIC); rearrangement at 9q13, 10p11 (MAP3K8, SHH3BP1), Xq13, 6q15, 22q13; der(10)t(10;17)(p11;q11); t(1;6)(q21;q25); and trisomy 2. More recently, there has been interest in whether SEF is related to low-grade fibromyxoid sarcoma, and if they share common translocations t(7;16)(132-34;p11) FUS-CREBL2 or t(11;16)(p11;11) FUS-CREB3L1). A limited number of SEFs have been shown to share one of these translocations associated with low-grade fibromyxoid sarcoma, suggesting that SEF may be within the spectrum of this tumor category.

SEF tends to be regarded as a intermediate to high-grade sarcoma with high local recurrence (50%) and metastatic (45-85%) rates. Metastatic sites include lung, bone, soft tissues, breast, pericardium, brain, pleura and lymph nodes. Bone invasion is not uncommon and may confuse this tumor with a bone-derived neoplasm. Clinical management is based upon complete excision of the tumor, usually followed by radiation and chemotherapy. Mortality tends to be high (60%) within 13-87 months following diagnosis. One-third are alive with disease and less than 10% are disease free. There is no correlation with mitotic activity, necrosis, vascular of bone invasion or areas of conventional fibrosarcoma in the tumor.

**Sclerosing Rhabdomyosarcoma**

Sclerosing rhabdomyosarcoma (SRMS) is a recently described matrix-rich rhabdomyosarcoma variant with hyalinized stroma. SRMS may be mistaken for osteosarcoma, chondrosarcoma or angiosarcoma (when a prominent pseudovascular pattern is present). This tumor has been described in both children and adults, with the majority of cases described in the pediatric age group (4 months to 18 years of age, mean 7.8 years of age). Sites of involvement are the head and neck, extremities, retroperitoneum, pelvis, sacrum and scrotum.

The tumor may not be recognized as being in the rhabdomyosarcoma family and a misdiagnosis of osteosarcoma, chondrosarcoma or angiosarcoma may be made. The tumor has ovoid to polygonal tumor cells embedded in an osteoid to chondroid matrix or within a hyalinized matrix. The tumor cells do not appear to be spindled or strap-like. Immunocytochemistry is helpful with the tumor cells expressing MyoD1, myogenin, desmin, SMA and MSA. Electron microscopy can provide the primary diagnosis or confirmatory evidence of myogenic origin of the tumor. Myofilaments, z-band material, dense bodies, and thick and thin myofilaments may be identified within occasional tumor cells. Many of the tumor cells lack tumor-defining features.
Cytogenetics and molecular studies have been done in a limited number of cases. However, an alveolar rhabdomyosarcoma translocation has been described in some of the tumors. There are some reports of genetic alteration at 10q22 (BMPR1A - bone morphogenic protein receptor 1 gene; PTEN; CD-Cowden gene; PJI - polyposis juvenile intestinal gene) and trisomy 18. It has also been noted with SNP genotyping that the tumors tend to have a highly aneuploid profile and MDM2/HMGA2 amplification at 12q13-15. The 12q13-15 amplification is well known in rhabdomyosarcoma and this regions harbors SAS, GLI, CDK4. MDM2 (associated with p53) and HMGA2 genes.

Treatment is based upon rhabdomyosarcoma-based therapy (chemotherapy, complete excision and potential radiation therapy). Local recurrence rate is approximately 20-25%, with a similar rate for metastatic disease (15-20%). Follow-up in these patients tend to be relatively short. However, outcome in these patients is dead of disease about 10%, alive with disease about 65%, and no evidence of disease about 25%.

**Congenital Ewing's Sarcoma**

Typically, small round cell tumors that are identified during the neonatal period are leukemias and neuroblastomas. Ewing sarcoma (peripheral primitive neuroectodermal tumors) are not usually in the initial differential diagnosis. This tumor is a rare finding and may be misdiagnosed in the neonate, especially because these tumors have variable degrees of neuroectodermal differentiation. This is understandable because the median age range for Ewing's sarcomas is 15 years of age, with 70% presenting before 20 years of age. About 15% of Ewing's sarcomas are diagnosed before 5 years of age. In general, congenital malignant tumors account for less than 2% of all childhood malignancies. The typical differential diagnosis for congenital tumors include teratoma, rhabdomyosarcoma, neuroblastoma, hemangioma, other vascular tumors, leukemia, lymphoma and lymphatic malformations.

Only a small number of Ewing's sarcomas have been reported as being congenital or discovered during the neonatal period. There are a total of 22 cases reported in the literature. The sites of involvement are quite variable and often times involve the head and neck region and soft tissues. Metastatic disease at time of diagnosis has been reported in about 40% of cases with skin, lymph nodes and brain being the sites involved. Initial diagnoses were alveolar rhabdomyosarcoma, embryonal rhabdomyosarcoma, undifferentiated sarcoma, malignant peripheral nerve sheath tumor, neuroepithelioma and Ewing's sarcoma/peripheral neuroectodermal tumor. Treatment has been variable from sarcoma-based chemotherapy with or without radiation to complete surgical excision alone. Unfortunately, few neonates survive this tumor with 15 of 22 succumbing to disease from 1 month to 2 years following diagnosis.

Ewing's sarcoma histopathology is quite variable with some demonstrating neuroectodermal differentiation as noted by immunocytochemical expression of chromogranin, synaptophysin, and NSE. The more typical pattern is a small round cell tumor with lack of differentiation and CD99 expression (classic type of Ewing's sarcoma), which may be confused with other small round cell tumors. Electron microscopy provides support for the diagnosis of Ewing's sarcoma family of tumors with bland nuclear morphology, cytoplasmic glycogen accumulations and rare neurosecretory granules, and lack of neurite-like cell processes. Rudimentary intercellular junctions are also present which will eliminate a concern for a hematolymphoid neoplasm. Cytogenetics and molecular diagnostics are important in order to identify translocations associated with Ewing's sarcoma, as well as to identify EWS gene rearrangement by FISH and potentially novel EWS gene translocations with new partner genes. The translocations that may be seen in Ewing's sarcoma include EWS gene translocations (EWS-FLI1, EWS-ERG, EWS-ETV1, EWS-E1AF, EWS-FEV, EWS-ZNF278, EWS-POU5F1, EWS-SP3) and non-EWS gene translocations (FUS-ERG, FUS-FEV, CIC-DUX4, BRD4-NUT). This emphasizes the importance of submitting tissue for cytogenetic and FISH analyses, in addition to performing routine RT-PCR for the most common Ewing's sarcoma translocations.
(EWS-FLI in 85-95% of cases; EWS-ERG in 5-10% of cases). In fact, novel translocations are not uncommon in neonatal Ewing’s sarcoma. Two recent cases have shown EWS gene rearrangement with chromosome 20 [t(20;22)], with the novel partner gene(s) yet to be defined.

**Undifferentiated Neuroblastoma with ALK-1 Expression**

Neuroblastoma is a more common small round cell tumor derived from the neural crest that occurs in young children. The majority of children are less than 5 year of age at diagnosis and some are diagnosed during the neonatal period or in early infancy. This tumor has a wide spectrum of neural ectodermal differentiation from undifferentiated neuroblastoma to poorly differentiated neuroblastoma to differentiating neuroblastoma to ganglioneuroblastoma to ganglioneuroma. Neuroblastoma has the ability to differentiate “spontaneously” over time and with the “assistance” of chemotherapeutic intervention.

Typically, neuroblastoma is not a diagnostic dilemma because of the typical granular nuclear chromatin morphology, presence of neuropil and the formation of pseudorosettes. Immunocytochemical studies show that neuroblastoma tumor cells react with NB-84 with high sensitivity and specificity and with other neural markers including PGP9.5, NSE, chromogranin, synaptophysin, MAP and S100 protein. Vimentin is typically negative with neuroblastoma. MYC-N may be amplified in high-risk and unfavorable histology neuroblastomas. Aberrant reaction with immunocytochemical markers may prove to be misleading. Particularly difficult in diagnosis is the undifferentiated neuroblastoma that does not arise within the adrenal gland or in adjacent sympathetic nervous system chain or that is metastatic at the time of diagnosis without a known primary tumor. This may precipitate a "small round cell tumor workup" with several immunocytochemical markers to rule out other tumors. Electron microscopy can provide evidence for neuroectodermal differentiation readily in such tumors. These tumors possess neurite processes, rudimentary intercellular junctions and most importantly uniform round, small dense core neurosecretory granules.

A major confusing factor with undifferentiated neuroblastomas is their immunoreactivity with ALK1, characteristic for anaplastic large cell lymphoma and inflammatory myofibroblastic tumor. The undifferentiated nature of this small round cell tumor and ALK1 positivity have lead to an aberrant diagnosis of anaplastic large cell lymphoma in several cases. Interesting, genetic alterations of the anaplastic lymphoma kinase (ALK) have recently been identified in both familial and sporadic forms of neuroblastoma. These alterations include activating mutations in the kinase domain as well as DNA amplification of the ALK gene, and occur in many of the familial forms of neuroblastoma and in approximately 15% of sporadic cases. This provides evidence for an additional genetic etiology of neuroblastoma, which leads to uncontrolled ALK downstream signaling. In a certain proportion of neuroblastomas, this also provides the potential for a previously unrecognized therapeutic opportunity in neuroblastoma by targeting ALK kinase using blocking antibodies or small molecule kinase inhibitors. If a small round cell tumor of childhood expresses ALK by immunocytochemistry, ultrastructural examination can provide evidence for or against the diagnostic of lymphoma vs. neuroblastoma. This recent finding of ALK overexpression in neuroblastoma is important in avoiding misdiagnosis in neuroblastic tumors.

**Acute Megakaryoblastic Leukemia with [t(1;22)] Mimicking Neuroblastoma**

Acute megakaryoblastic leukemia with t(1;22) is a rare form of acute myeloid leukemia and represents only about 1% of cases. It is restricted to infants and children up to 3 years of age, with the majority of cases occurring during the 1st 6 months of life (median age 4 months). Most present with marked organomegaly, in particular hepatomegaly. These patients may also have anemia and thrombocytopenia. Due to the rarity of AMKL, the clinician’s suspicion in a
neonate or infant is most likely neuroblastoma with liver and bone marrow involvement (stage IVS or stage IV neuroblastoma).

The pathologist may receive needle core or wedge biopsies of the liver for diagnostic purposes. The histopathologic findings are those of an undifferentiated small round cell tumor that lacks histopathologic features of a specific childhood malignancy. The tumor cells infiltrate the liver diffusely and replace the hepatocytes with retention of the bile ducts. There may be "normal" hematopoiesis typically seen in the neonate or infant. The findings with the liver biopsy are typical for an undifferentiated neuroblastoma with a neonate or infant with Stage IV or Stage IVS neuroblastoma. With a high suspicion for neuroblastoma, immunocytochemical studies for neuroblastoma are usually performed and these will show NB-84 (neuroblastoma marker) and NSE positivity with or without PGP9.5 reactivity. With such immunocytochemical results, an errant diagnosis of neuroblastoma may be rendered. With undifferentiated neuroblastoma or small round cell tumors, it is advisable to perform electron microscopy to confirm the diagnosis. In such cases, the lack of neurosecretory granules, neurite-like processes and rudimentary cell junctions will disprove neuroblastoma as an appropriate diagnosis. Tissue submitted for cytogenetics will provide FISH results indicating nonamplification on MYC-N, which is common in neuroblastomas in neonates and infants. This will not provide helpful information in disproving a diagnosis of neuroblastoma. Translocation detection for t(1;22)(p13;q13) associated with RBM15-MKL1 genes (also known as OTT-MAL) will refute a diagnosis of neuroblastoma; however, this result will not be available for some time, and could result in misdiagnosis with initiation of neuroblastoma therapy prior to providing the appropriate diagnosis of AMKL with t(1;22). Electron microscopic examination of these undifferentiated tumors involving the liver, soft tissue or other organs can redirect the diagnostic workup toward a hematolymphoid neoplasm. Immunocytochemistry for platelet glycoproteins (CD41 glycoprotein IIb/IIIa; CD61 glycoprotein IIIa; CD42 glycoprotein 1b) and myeloid markers (CD12, CD33) will provide evidence for the diagnosis of AMKL while awaiting cytogenetic confirmation of the t(1;22) translocation. In addition, FISH studies may be requested for the gene partners in the t(1;22) translocation. Bone marrow biopsy to identify AMKL, rather than neuroblastoma infiltration of the bone marrow, as responsible for the anemia and thrombocytopenia may also be initiated. Considerable bone marrow fibrosis may also be present with AMKL and may not demonstrate AMKL by microscopic or flow cytometric examination.

In the past, prognosis for this AMKL was considered poor; however, current intensive AML chemotherapy has resulted long-term disease free survival.

**Congenital Self-Healing Langerhans Cell Histiocytosis**

Although first described some 3 decades ago by Hashimoto and Pritzker, the least recognized LCH type is congenital self-healing LCH (CSDLCH). This entity is also known within the literature as congenital self-healing reticulohistiocytosis and as Hashimoto-Pritzker’s histiocytosis. It has been estimated that as many as 10% of cases of LCH may be this type. Typically with CSDLCH, a neonate or young infant presents with asymptomatic cutaneous lesions that are violaceous red to brown, firm nodules. The lesions may also resemble more classic LCH with a papular, vesicular or macular appearance. The most common sites are the face, scalp, trunk, extremities, soles and palms. The lesions may arouse suspicion of more serious neonatal diseases, such as congenital leukemia, neuroblastoma or infectious processes. Multiple lesions are more common than solitary ones. Typically the lesions tend to involute spontaneously over several months, with some lesions being replaced by hypopigmented or hyperpigmented macules. Only a single case of visceral and bone involvement has been reported, and the lesions in this case resolved with a short course of systemic steroid administration. With the exception of this unique CSDLCH case, all other infants have had their lesions undergo spontaneous resolution without interventional therapy.
Although this form of LCH is considered to be benign, two cases have had recurrences, with one case requiring systemic chemotherapy when a bone lesion was identified. This emphasizes the need for long-term follow-up to evaluate the infant for new lesion development.

In contrast to the spontaneous involution occurring with CSHLCH, the other classic LCH types reported as congenital or neonatal cases have not had such a benign course. In all cases, the presenting symptom was skin lesions, but almost three-quarters of the neonates and infants had other organs involved when a diagnosis was finally made. Bone was most commonly involved followed by a variety of other organs. Two or more organ systems were involved in 86% of neonates. It is interesting to note that all children had lesions at birth or during the first month of life, but the mean age was slightly over 3 months when the diagnosis was actually made. This emphasizes that classic LCH may mimic many benign conditions seen in the neonatal period and infancy, especially eczema. Over 70% of the classic LCH-affected neonates required treatment, ranging from surgery to chemotherapy and radiation. It is interesting to note that about 30% required no treatment. These neonates were those with skin disease only, and this raises the possibility that these neonates may actually have had CSRLCH instead.

The histopathologic features of CSHLCH are identical to those described for classic LCH, except for unique ultrastructural findings. The lesion is composed of an admixture of Langerhans cell histiocytes, indeterminate cells of a dendritic lineage, interdigitating cells, T-cell lymphocytes, eosinophils and macrophages. Langerhans cell histiocytes possess reniform, grooved or deeply indented nuclei with relatively abundant amphophilic to eosinophilic cytoplasm. Occasionally giant cells representing either fusion of macrophages or LHC cells may be found. Necrosis and eosinophilic abscesses are not uncommon. The histopathologic appearance of LCH varies from tissue type to tissue type. Occasionally, LCH lesions take on the form of epithelioid granulomas. With CSHLCH, the cutaneous lesion more typically involves the deep dermis in a nodular pattern, with sparing of the epidermis. In all LCH lesions, mitotic activity may be present, but typically is moderate to low.

According to the Histiocyte Society’s LCH criteria, a tentative diagnosis of LCH may be made on routinely stained tissue sections. In order to provide a definitive diagnosis of LCH, CD1a or CD207 immunoreactivity and/or Langerhans cell granules (LC granules, Birbeck granules, Birbeck-Broadbent granules) are required.

A monoclonal antibody against a type II transmembrane protein has become commercially available, and interacts with a specific 40kD protein restricted to Langerhans cells and Langerhans cell histiocytes. This antibody is known as Langerin (CD207). Langerin is located on the cell membrane of Langerhans cells and is a potent inducer of membrane superimposition and "zippering" leading to LC (Birbeck) granule formation in these cells. It is rapidly internalized from the cell surface into the Birbeck granule.

The ultrastructural morphology of LC (Birbeck) granules is well known and provides the “gold standard” in the diagnosis of Langerhans cell histiocytosis. All LCH types demonstrate the well-described LC (Birbeck) granule morphology. The LC granule is characterized as either a rod or racket-shaped cytoplasmic structures of 200 to 400nm in length with a constant diameter of 33nm. The LC granules are described as pentilaminar structures resembling a "zipper", because of a central striated membrane and a double outer sheath. LC granules are more readily detected in early LCH lesions than long-standing ones. They tend to be rare in LCH involving the liver, gastrointestinal tract and the spleen. LC granules are present in a variable numbers of Langerhans cell histiocytes within a lesion. Other ultrastructural features found with LCH include multivesicular bodies, curvilinear membranous formations, tubuloreticular inclusions, and cylindrical confronting cisterna. These are not specific to LCH and are found to a variable extent with other types of lesions.

Congenital self-healing LCH may only be differentiated from the other forms of LCH by ultrastructural examination. CSHLCH will immunoreact with CD1a, CD207 (Langerin), S100
and protein in the same manner as the other types of classical LCH. Ultrastructural evaluation of a suspected CSHLCH nodule allows for definitive confirmation of this diagnosis. CSHLCH contains lesion-defining laminated dense bodies, characterized by concentric myelin-like material within dense bodies. These characteristic dense bodies occur in relatively large numbers, while LC (Birbeck) granules are present in a minority of lesional cells (<30%). In addition, non-laminated dense bodies may also be noted frequently. The origin of the dense bodies is not known. The identification of laminated dense bodies in LCH affecting a neonate or infant may allow for reassurance regarding the clinical suspicion of congenital self-healing LCH versus a more aggressive form of LCH that requires chemotherapy.

**Congenital Melanoma**

Melanoma rarely affects children with only 1.3% of melanoma affecting individuals below the age of 20 years and in 0.9% of those below 15 years of age (childhood melanoma). Congenital (in utero and present at birth) and infantile (birth to 1 year) melanoma are extremely rare. Congenital and infantile melanomas may arise from transplacental metastases (13%), medium to large congenital melanocytic nevi (57%) and other nevi (30%). The most common sites are head and neck (43%), extremities (26%), trunk (23%) and skin (9%). Males were more frequently involved than females (2.8M:1.0F).

Particularly troublesome is when there is no apparent melanocytic nevus with the neonate, infant or young child and the tumor presents as a soft tissue mass with no melanin pigment and lack of typical melanoma cell features. Melanomas in childhood and infancy are unusual histopathologic features and frequently do not fall within the spectrum of conventional adulthood melanomas. Small cell variants of congenital and infantile melanoma have been reported and are particularly troublesome. These are often times confused with lymphoma, soft tissue leukemic involvement, neuroblastoma and other childhood small round cell tumors. In particular, electron microscopy may be of great importance in providing the origin of the tumor, especially when melanoma is not a consideration by the clinician. Once premelanosomes are identified, the pathologist will then provide an accurate diagnosis and may perform additional immunocytochemical studies (S100, HMB45, MelanA, Mart1, etc.) to further confirm melanoma as a diagnosis.

With congenital and infantile melanoma, about 45% of those affected die of disease from days to 18 months after diagnosis. Long-term survival data are not available on most of the neonates and infants indicated as alive in case reports and small case series in the literature. However, clinical follow-up in those reported as alive following a diagnosis of congenital or infantile melanoma has varied from 1 year to 20 years.
Table: Triaging of Tissue for Pediatric Tumors

Small Round Cell Tumors
- Frozen tissue with cryopreservative for intraoperative diagnosis
- Cytologic, scrape and squash imprints for intraoperative diagnosis
- Formalin-fixed tissue for routine histopathology, immunocytochemistry, *in situ* hybridization and reverse transcriptase polymerase chain reaction (RT-PCR) evaluation
- Glutaraldehyde-fixed tissue for electron microscopy
- Fresh tissue in tissue culture media for cytogenetics and molecular studies, and tissue cultures
- Frozen tissue without cryopreservative for molecular studies, gene rearrangement, and microarray gene analysis
- Fresh tissue for flow cytometric analysis for DNA ploidy or cell surface markers
- Fresh tissue for biochemical analyses of tumor specific products
- Cytologic imprints of neoplastic tissue
  - Cytogenetic interphase studies
  - Fluorescent *in situ* hybridization for cytogenetics (FISH)
  - Special stains and immunocytochemical phenotyping
- Alcohol-fixed tissue for improved cytoplasmic glycogen preservation, immunocytochemistry (requiring such fixation) and microarray gene analysis

Tissue with Inflammatory and/or Infectious Histopathologic Features
- Fresh tissue for microbiologic studies
  - Bacterial (aerobic and anaerobic) cultures
  - Mycologic cultures
  - Acid-fast bacillus culture and RT-PCR
- Fresh tissue for virology studies
  - Viral cultures
  - RT-PCR for rapid viral identification or for viruses that cannot be cultured
  - Molecular virology studies
- Frozen tissue without cryopreservative for molecular microbiology and virology studies, including RT-PCR study
<table>
<thead>
<tr>
<th>Antibody</th>
<th>Antigen/CD</th>
<th>Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-vimentin</td>
<td>Vimentin</td>
<td>Generic anti-intermediate filament; quality control; reactive with most mesenchymal cells</td>
</tr>
<tr>
<td><strong>Hematopoietic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-LCA</td>
<td>CD45 (all subunits)</td>
<td>Common leukocyte antigen; no lineage specificity, unlike CD45RA, RB, and RO</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td></td>
<td>Myeloid cells and tumors</td>
</tr>
<tr>
<td>Muramidase</td>
<td>Lysozyme</td>
<td>Myeloid cells and tumors, histiocytic disorders, macrophages, fibrohistiocytic tumors</td>
</tr>
<tr>
<td>LeuM1</td>
<td>CD15</td>
<td>T cells, Hodgkin's disease</td>
</tr>
<tr>
<td>Ki-1</td>
<td>CD30</td>
<td>Hodgkin's disease, anaplastic large cell lymphoma</td>
</tr>
<tr>
<td>ALK-1</td>
<td>ALK-1</td>
<td>Anaplastic large cell lymphoma, inflammatory Myofibroblastic Tumor, Neuroblastoma (certain cases)</td>
</tr>
<tr>
<td>NPM</td>
<td>P80NPM/ALK</td>
<td>Anaplastic large cell lymphoma</td>
</tr>
<tr>
<td>L26</td>
<td>CD20</td>
<td>B cells, rhabdomyoblasts</td>
</tr>
<tr>
<td>UCHL</td>
<td>CD45RO</td>
<td>T cells</td>
</tr>
<tr>
<td>T3</td>
<td>CD3</td>
<td>T cells; possibly superior to UCHL1</td>
</tr>
<tr>
<td>T6</td>
<td>CD1a</td>
<td>Langerhans cell histiocytosis and Langerhans cells</td>
</tr>
<tr>
<td>Langerin</td>
<td>CD207</td>
<td>Langerhans cell histiocytosis, Langerhans cells</td>
</tr>
<tr>
<td>TdT</td>
<td>Terminal deoxynucleotidyl transferase</td>
<td>Lymphoblastic lymphoma, ALL T cell</td>
</tr>
<tr>
<td>EBER-1</td>
<td>Epstein-Barr encoded RNA-1</td>
<td>EBV-associated B-cell lymphoma, Hodgkin's lymphoma, lymphoproliferative disease (including posttransplantation), smooth muscle tumors in HIV/AIDS</td>
</tr>
<tr>
<td>Fascin</td>
<td>55K2</td>
<td>Dendritic cell sarcoma, dendritic cells</td>
</tr>
<tr>
<td>Factor XIIa</td>
<td>Factor XIIa</td>
<td>Dendritic cell sarcoma, dendritic cells</td>
</tr>
<tr>
<td>Ham 56, KP-1, PGM1, MAC387</td>
<td>CD68</td>
<td>Histiocytic disorders, macrophages, fibrohistiocytic tumors, sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease)</td>
</tr>
<tr>
<td>Cathepsin B</td>
<td></td>
<td>Histiocytic disorders, macrophages, fibrohistiocytic tumors</td>
</tr>
<tr>
<td><strong>Neural</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSE</td>
<td></td>
<td>Neural antigen, but poorly specific</td>
</tr>
<tr>
<td>NB-84</td>
<td>Neuroblastoma</td>
<td></td>
</tr>
<tr>
<td>TrkA</td>
<td>Neuroblastoma</td>
<td></td>
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<tr>
<td>PGP 9.5</td>
<td>Neuroblastoma</td>
<td></td>
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<tr>
<td>TH</td>
<td>Tyrosine hydroxylase</td>
<td>Neuroblastoma</td>
</tr>
<tr>
<td>NFTP</td>
<td>Neuronal cells, tumors (e.g., neuroblastoma)</td>
<td></td>
</tr>
<tr>
<td>GFAP</td>
<td>Gial cells (gliosis, gliomas)</td>
<td></td>
</tr>
<tr>
<td>S-100</td>
<td>S-100A</td>
<td>Nerve sheath tumors, melanoma, neuroblastoma, Langerhans cells</td>
</tr>
<tr>
<td>Chromogranin</td>
<td>Neuroblastoma, neural tumors</td>
<td></td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Neural tumors</td>
<td></td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Medullary carcinoma of thyroid</td>
<td></td>
</tr>
<tr>
<td>P30/32MIC2</td>
<td>CD99, O13, 12E7, HBA71</td>
<td>Ewing sarcoma family of tumors, Lymphoblastic lymphoma, lymphocytes, endothelial cells</td>
</tr>
<tr>
<td>FLI-1</td>
<td></td>
<td>Ewing sarcoma family of tumors, lymphoblastic lymphoma, lymphocytes</td>
</tr>
<tr>
<td>Leu7</td>
<td>CD57</td>
<td>Peripheral nerve sheath tumors, Schwann cells, neuroblastomas</td>
</tr>
<tr>
<td>P75NTR</td>
<td></td>
<td>Nerve sheath tumors</td>
</tr>
<tr>
<td>Neural cell adhesion</td>
<td>NCAM</td>
<td>Neural tumors, neuroendocrine cells, neuroblastic</td>
</tr>
<tr>
<td>molecule</td>
<td>cells, natural killer T cells, sarcomas</td>
<td></td>
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<tr>
<td><strong>Myogenic</strong></td>
<td></td>
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<tr>
<td>Desmin</td>
<td>Myogenic and myofibroblastic tumors</td>
<td></td>
</tr>
<tr>
<td>MSA</td>
<td>Myogenic tumors</td>
<td></td>
</tr>
<tr>
<td>Myoglobin</td>
<td>Skeletal muscle tumors</td>
<td></td>
</tr>
<tr>
<td>MyoD</td>
<td>Skeletal muscle tumors (specific)</td>
<td></td>
</tr>
<tr>
<td>Myogenin</td>
<td>Skeletal muscle tumors (specific)</td>
<td></td>
</tr>
<tr>
<td>MRF-4-herculin/myf6</td>
<td>Skeletal muscle tumors (specific)</td>
<td></td>
</tr>
<tr>
<td>Caldesmon</td>
<td>Myogenic tumors</td>
<td></td>
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<td>Cáncer, sarcoma, carcinoma, células de células germinales, tumores hepáticos, tumores de células epiteliales, sarcoma de células rhabdóides, tumores teratóides-atípicos-rhabdóides</td>
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<td><strong>Vascular</strong></td>
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<td>TRAIL</td>
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<td>Survivin</td>
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<td>P27 (kip1)</td>
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<td>Cyclin E</td>
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<td>PTEN</td>
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<td>Antibody</td>
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<td>Leukemia, lymphoma</td>
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<tr>
<td>Myogenin</td>
<td>Rhabdomyosarcoma</td>
<td></td>
</tr>
<tr>
<td>Desmin</td>
<td>Rhabdomyosarcoma, desmoplastic small round cell tumor</td>
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</tr>
<tr>
<td>Muscle-specific actin CD99 (MIC2)</td>
<td>Ewing sarcoma, lymphoma, leukemia</td>
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<td>Pancytokeratin</td>
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<tr>
<td>Alpha fetoprotein</td>
<td>Hepatoblastoma, endodermal sinus tumor (yolk sac tumor)</td>
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<tr>
<td>CD1a</td>
<td>Langerhans cell histiocytosis</td>
<td></td>
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<tr>
<td>CD207 (Langerin)</td>
<td>Langerhans cell histiocytosis</td>
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</tr>
<tr>
<td>CD30 (Ki-1, Ber-H2)</td>
<td>Anaplastic large cell lymphoma</td>
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<td>ALK-1</td>
<td>Anaplastic large cell lymphoma, inflammatory myotubroblastic tumor, Neuroblastoma (certain cases)</td>
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<tr>
<td>INI1/SMARCB1</td>
<td>Rhabdoid, Epithelial Sarcoma, Atypical Teratoid/HabdoId Tumor, Small Cell (Undifferentiated), Hepatoblastoma, Subset of Extraskeletal Myxoid Chondrosarcoma</td>
<td></td>
</tr>
<tr>
<td>TLE1</td>
<td>Synovial Sarcoma</td>
<td></td>
</tr>
<tr>
<td>Vimentin</td>
<td>Antigen Maintenance Determination, Rhabdoid Tumor, Fibrosarcoma, Spindle Cell Tumors, Mesenchymal Tumors, and Absent in Neuroblastoma,</td>
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</tr>
<tr>
<td>TFE3</td>
<td>Xp11.2 renal tumors, alveolar soft parts sarcoma</td>
<td></td>
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</table>

**Immunoreactivity of Pediatric Tumors**

<table>
<thead>
<tr>
<th>Neuroblastoma Neuroblastic</th>
<th>Stromal Cell</th>
<th>Cell Surface</th>
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<tbody>
<tr>
<td>NB84</td>
<td>Protein gene product 9.5</td>
<td>S-100 protein</td>
</tr>
<tr>
<td>NSE</td>
<td>Microtubule associated protein (MAP 1/2)</td>
<td>Glial fibrillary protein</td>
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<tr>
<td>Dopamine</td>
<td>Vasoactive intestinal protein</td>
<td>Myelin basic protein</td>
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<td>Neurofilament triple protein</td>
<td>ALK-1 (certain cases)</td>
<td>Ganglioside G-D2</td>
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<td>Rhabdomyosarcoma</td>
<td>Ewing Sarcoma/Peripheral Primitive Neuroectodermal Tumor</td>
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<tr>
<td>Myogenin</td>
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<td>CD99</td>
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<tr>
<td></td>
<td>S-100 protein</td>
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</tbody>
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**Table**

**IMMUNOCYTOCHEMISTRY APPROACH TO SMALL ROUND CELL TUMORS OF CHILDHOOD**

**Undifferentiated Small Round Cell Tumor Immunocytochemistry Panel**
<table>
<thead>
<tr>
<th>Desmoplastic Small Round Cell Tumor</th>
<th>Rhabdoid Tumor (Renal/Extrarenal)</th>
<th>Synovial Sarcoma</th>
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<tbody>
<tr>
<td>Desmin</td>
<td>Vimentin</td>
<td>EMA</td>
</tr>
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<td>Cytokeratin</td>
<td>W T-1</td>
<td>Cytokeratin</td>
</tr>
<tr>
<td>NSE</td>
<td>Leu-7 (CD57)</td>
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<td>Epithelial membrane antigen (EMA)</td>
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<table>
<thead>
<tr>
<th>Wilms' Tumor (Nephroblastoma)</th>
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<tbody>
<tr>
<td>Tubular Epithelium</td>
<td>Blastaema</td>
<td>Stroma</td>
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<td>Desmin</td>
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<tr>
<td>W T-1</td>
<td>S-100 protein</td>
<td>Cytokeratin</td>
</tr>
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<td>Alpha fetoprotein</td>
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</tr>
<tr>
<td></td>
<td>Beta-HCG</td>
<td>CEA</td>
</tr>
<tr>
<td></td>
<td>Beta-Catenin</td>
<td>Beta-Catenin</td>
</tr>
<tr>
<td></td>
<td>Absence of INI1/SMARCB1</td>
<td></td>
</tr>
</tbody>
</table>

| Medulloblastoma (Primitive Neuroectodermal Tumor) |                            |                  |
| Neuron specific enolase            | S-100 protein                | Synaptophysin    |
| Ciliary fibrillary acidic protein  | Nestin                       | Neuron-specific  |
| Microtubule-associated protein     | Tubulin                      | enolase          |
|                                  |                               |                 |

<table>
<thead>
<tr>
<th>Leukemia/Lymphoma</th>
<th>Myofibroma/Myofibromatosis</th>
<th>Embryonal Sarcoma of Liver</th>
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<tr>
<td>LCA, CD99 (MIC2)</td>
<td>Smooth muscle actin</td>
<td>Vimentin</td>
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<td>CD79a, CD 19/20</td>
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<td>CD3/CD4/CD8/CD45RO (T cell)</td>
<td>Collagen, Beta-Catenin</td>
<td>Alpha-1-antitrypsin</td>
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<td>ALK-NPM, CD30, epithelial membrane antigen (anaplastic large cell lymphoma)</td>
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<table>
<thead>
<tr>
<th>Germ Cell Tumors</th>
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<td>Beta-huma chionic gonadotropin</td>
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<td>Placental alkaline phosphatase</td>
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<td>Intratubular Germ Cell Neoplasia</td>
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<td>Cytokeratin</td>
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<td>Pleuropulmonary Blastoma</td>
<td>Mesenchymal Chondrosarcoma/Chondrosarcoma</td>
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</tr>
<tr>
<td>Vimentin</td>
<td>S-100 protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desmin</td>
<td>Vimentin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle-specific actin, DICER1</td>
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<tr>
<td>Langerhans Cell Histiocytosis</td>
<td>Small Cell Osteosarcoma</td>
<td>Malignant Peripheral Nerve Sheath Tumor</td>
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<td>CD1a</td>
<td>Vimentin</td>
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<tr>
<td>CD207 (Langerin)</td>
<td>pHb</td>
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<td>S-100 protein</td>
<td>Collagen type IV</td>
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<td>Factor XIIIa</td>
<td>S-100 protein</td>
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<td>CD68</td>
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<td>Myxoid Liposarcoma/Myxoid Lipoblastoma</td>
<td>Dysplastic Nevus/Cutaneous Melanoma/Clear Cell Sarcoma of Soft Tissues</td>
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<tr>
<td>S-100 protein</td>
<td>S-100 protein</td>
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<td>HMB-45</td>
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<td>Melan-A</td>
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<td>Tyrosinase</td>
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<td>MTF-1</td>
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<td>Proliferation Markers</td>
<td>Cyclin dependent kinases</td>
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<td>Mib-1 (Ki-67)</td>
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<tr>
<td>PCNA</td>
<td>Cyclins (A, B, D1, D2)</td>
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<td>p53</td>
<td>Bcl-2</td>
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<td>Antibody Panel</td>
<td>Tumor Type</td>
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<tr>
<td>Myogenin</td>
<td>Rhabdomyosarcoma, DSRCT</td>
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<td>CD99 (MIC2)</td>
<td>Ewing sarcoma family of tumors</td>
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<td>INI1/SMARCB1</td>
<td>Rhabdoid Tumor, Epithelioid Sarcoma, Atypical Teratoid/Rhabdoid Tumor, Small Cell (Undifferentiated) Hepatoblastoma, Subset of Extraskeletal Myxoid Chondrosarcoma</td>
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<td>Synovial Sarcoma</td>
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<tr>
<td>Vimentin</td>
<td>Antigen preservation, Rhabdoid Tumor, Fibrosarcoma, Myofibroma, Spindle Cell Tumors, Mesenchymal Tumors, But Absent in Neuroblastoma</td>
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<tr>
<td>TFE3</td>
<td>Xp11.2 Renal Tumors, Alveolar Soft Parts Sarcoma</td>
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</tbody>
</table>

**Antibodies for Defining Cell of Origin**

Myogenic: Desmin, myogenin, MyoD1, muscle specific actin
Neural: NB84, NSE, S-100 protein, ALK-1 (certain cases)
Hematopoietic/lymphoid: LCA, myeloperoxidase
Germ cell: Alpha fetoprotein, PLAP, beta-HCG, keratin
Neural crest: S-100 protein, HMB-45, CD99, NCAM
Mesenchymal: Vimentin, smooth muscle actin

**Aberrant Immunoreactivity**

Vimentin: Neuroblastoma (absence of vimentin)
Cytokeratin: Ewing sarcoma family of tumors, rhabdomyosarcoma, lymphoma, lymphoid leukemia
CA-125: Desmoplastic small round cell tumor
Desmin: Ewing sarcoma family of tumors, malignant peripheral nerve sheath tumor, rhabdoid tumor
Muscle-specific actin: Ewing sarcoma family of tumors, malignant peripheral nerve sheath tumor, rhabdoid tumor
Smooth muscle actin: Small cell osteosarcoma
Epithelial membrane antigen: Ewing sarcoma family of tumors, rhabdomyosarcoma, lymphoma, lymphoid leukemia
NB84: Ewing sarcoma family of tumors, desmoplastic small round cell tumor, megakaryocytic leukemia, bile duct tumors
S-100 protein: Rhabdomyosarcoma, small cell osteosarcoma
Neuron-specific enolase: Rhabdomyosarcoma, rhabdoid tumor
TrkA: Ewing sarcoma family of tumors, rhabdomyosarcoma
CD99: Rhabdomyosarcoma, desmoplastic small round cell tumor, synovial sarcoma
CD19/20: Rhabdomyosarcoma
Leu7 (CD57): Rhabdomyosarcoma, small cell osteosarcoma
CD68: Rhabdomyosarcoma, malignant peripheral nerve sheath tumor
LeuM1 (CD15): Desmoplastic small round cell tumor
ALK-1: Neuroblastoma (certain cases)
CD34: Malignant peripheral nerve sheath tumor
<p>| TABLE |
| ULTRASTRUCTURAL FEATURES FOR SMALL ROUND CELL TUMORS OF CHILDHOOD |
| Rhabdomyosarcoma |
| Z-bands |
| Thick and thin filaments |
| Myosin-ribosome complexes |
| Myotubules/myofilaments |
| Basement membrane |
| Monoparticulate glycogen |
| Intercellular junctions, rudimentary |
| Lymphoma |
| Polyribosomes |
| Lack of intercellular junctions |
| Paucity of organelles |
| Wilms', Blastemal Predominant |
| Thick, flocculent basement membrane |
| Cell junctions, well developed |
| Lumens |
| Microvilli and cilia |
| Basilar infolding |
| Endodermal Sinus Tumor |
| Nonmembrane bound |
| Spheroidal inclusions |
| Membrane bound inclusions |
| Congenital Infantile Fibrosarcoma |
| Granular extracellular matrix |
| Extracellular and intracellular |
| Collagen |
| Intercellular junctions, rudimentary basal lamina |
| Dilated rough endoplasmic reticulum |
| Hepatoblastoma |
| Intercellular junctions |
| Canalculi with microvilli |
| Smooth and rough endoplasmic reticulum |
| Desmoplastic Small Round Cell Tumor |
| Mesenchymal, rhabdoid, epithelial, and neuroblastic/neural features neurosecretory dense core granules |
| Intermediate filament whirls |
| Small glycogen lakes |
| Focal basal lamina |
| Rudimentary cell junctions |
| Neuroblastoma |
| Neurites |
| Cell processes with fine filaments |
| Neurosecretory granules |
| Intercellular junctions, rudimentary |
| Synaptic-like structures |
| Ewing Sarcoma/PNET |
| Focal glycogen aggregates |
| Neurites, blunted |
| Pleomorphic neurosecretory granules in PNET |
| Intercellular junctions, rudimentary |
| Rhabdoid Tumors &amp; Atypical Teratoid/Rhabdoid Tumors |
| Intermediate filament whirls with entrapped organelles |</p>
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<tr>
<th>Intercellular junctions, rudimentary</th>
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<td><strong>Clear Cell Sarcoma of Soft Parts/Melanoma</strong></td>
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<td>Melanosomes/premelanosomes</td>
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<td>Renal Tubulopapillary Neoplasms with Xp11.2 (TFE3) translocations</td>
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<td>Ewing’s Sarcoma/Primitive Neuroectodermal Tumor</td>
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<td>Non-EWS gene translocations</td>
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<td>Cardiomyopathy</td>
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<td>Desmoplastic Small Round Cell Tumor</td>
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<td>(Clear Cell Sarcoma of Soft Tissue)</td>
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<td>Cutaneous Melanoma and Dysplastic Nevus</td>
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<td>Dermatofibrosarcoma Protuberans and Giant Cell Fibroblastoma</td>
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<td>Low-Grade Fibromyxoid Sarcoma</td>
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INTRODUCTION

‘Avoiding pitfalls’ is not the same as ‘avoiding errors’, since errors can result from simple ignorance, technical error or incompetence. Although there are various definitions which are promulgated, the term ‘pitfall’ generally refers to unexpected or unforeseen difficulty or danger – although, as is generally well known, the original term referred to a hidden trap and a pathologist’s life seems hard enough without having to imply that specimens/slides actively seek to ensnare us! While all of us have ‘blind spots’ or areas of ignorance which may lead to misdiagnosis, in a brief overview such as this, it seems to me to make most sense to use the term ‘pitfalls’ to identify areas in which mistakes are commonly made due to unanticipated overinterpretation or else underinterpretation of specific morphologic features or immunophenotypic features which may lead to a misdiagnosis that has biologic or clinical significance. Mistaking one benign non-aggressive spindle cell neoplasm (e.g. myofibroma) for another (e.g. leiomyoma), while perhaps
regrettable or undesirable if we are seeking to do a perfect job, does not really qualify as a pitfall. Failing to recognize a rare or newly described entity represents a (generally understandable) gap in knowledge, not a pitfall. As a means of trying to validate what represents a morphologic diagnostic pitfall in soft tissue pathology, I have tried to identify the problems of this type which crop up most frequently in consultation material and a variety of examples are described briefly below.

‘WRONG’ TUMOR IN A GIVEN LOCATION

Failure to recognize that a particular morphologic pattern does not fit well with a given clinical/anatomic context or, conversely, the development of an otherwise usually easily recognized lesion at an unanticipated location can lead to a variety of often significant diagnostic problems. Examples are as follows:

**Angiolipoma**, which is usually diagnosed with ease, often seems to cause problems when arising in the breast, particularly if sampled by needle biopsy. Pathologists have a very low threshold for overinterpreting vascular lesions in the region of the breast as malignant – but important clues in angiolipoma are the absence of endothelial atypia or multilayering and the presence of distinctive microthrombi. When dealing with vascular lesions in the region of the ‘breast’ in general, it may also sometimes be helpful to understand whether the lesion was truly arising in breast parenchyma or whether instead the lesion is subcutaneous, since concern for malignancy relates mainly to parenchymal lesions.

**Myxoid liposarcoma** arises primarily in the limbs, particularly the lower limb, in the overwhelming majority of cases. Therefore, when making a diagnosis of myxoid liposarcoma at almost any other site, then the strong possibility that this might represent a metastasis from either an undisclosed or as yet undetected primary lesion in the limb should always be considered, particularly given the propensity of myxoid liposarcoma to metastasize to other soft tissue locations, very often, prior to the development of organ-based metastasis. Similarly, although liposarcoma is very common in the retroperitoneum, the large majority of such lesions are either well-differentiated or dedifferentiated and myxoid liposarcoma arising primarily at this site is very rare. However, a small but significant subset of well-differentiated liposarcomas in retroperitoneum may have a focally prominent myxoid stroma with thin-walled branching vessels and the main clue to the correct diagnosis is usually the presence of atypical spindle-shaped or
multinucleate tumor cells, not anticipated in myxoid liposarcoma. Immunostaining or FISH for MDM2 and CDK4 may also be helpful in this regard. Dedifferentiated liposarcoma may also have areas closely resembling myxofibrosarcoma but these should not be mistaken for myxoid liposarcoma which, again, very rarely shows the same degree of cytologic atypia or pleomorphism. Furthermore, the dedifferentiated areas should generally be non-lipogenic and lack lipoblasts.

**Malignant peripheral nerve sheath tumor** only very rarely arises primarily in the skin and, in that setting, is most often associated with a pre-existing neurofibroma. The large majority of cytologically atypical or malignant spindle cell neoplasms in the dermis which show positivity for S-100 protein, particularly if staining is diffuse, are in fact malignant melanomas, most often of spindle cell or desmoplastic type. Desmoplastic melanomas commonly lack any evident epidermal or junctional component, are frequently associated with a patchy lymphocytic infiltrate and may show neurotropism. Spindle cell and desmoplastic variants of melanoma are very commonly negative for second-line melanoma antigens such as HMB45 and MART-1 which may further increase the likelihood of a mistaken diagnosis of MPNST – however, melanomas show S-100 protein positivity in a much larger proportion of neoplastic cells than in most examples of MPNST.

**Fibroepithelial stromal polyp** of the vulvovaginal region is quite often mistaken for aggressive angiomyxoma, presumably because of concerns for the potentially more aggressive behaviour of the latter. However, as a simple rule of thumb, aggressive angiomyxoma almost never presents as an exophytic submucosal lesion but is almost invariably deep-seated, larger and notably more infiltrative. By contrast, stromal polyps are generally more cellular, lack any discernible margin and are characteristically exophytic. Immunohistochemistry cannot reliably distinguish between these entities.

**Sarcomas of spindle cell type**, other than the malignant mesenchymal component of a Phyllodes tumor, are extremely rare in the breast, albeit occasional such cases do occur. Nevertheless, whenever dealing with a cytologically atypical or malignant spindle cell neoplasm in the breast, in the absence of any epithelial-lined clefts to suggest Phyllodes tumor, then metaplastic (spindle cell) carcinoma is a far more likely possibility and, since any co-existent intra-ductal or invasive epithelial component is often not evident, immunostaining for several keratins and p63 will often be informative in this context.
BENIGN LESIONS WITH FREQUENTLY WORRISOME MORPHOLOGY

**Cellular benign fibrous histiocytoma** shows central necrosis in as many as 10-15% of cases and also very often extends into underlying subcutaneous adipose tissue. Neither of these features is indicative of malignancy. Attention should be paid to the mixed storiform and fascicular growth pattern, entrapment of hyaline collagen bundles and frequent overlying epidermal hyperplasia. The same guidelines are applicable to aneurysmal fibrous histiocytoma, which essentially represents cellular FH with prominent stromal hemorrhage and often increased numbers of stromal blood vessels.

**Atypical fibrous histiocytoma** (so-called ‘dermatofibroma with monster cells’) is very often mistaken for sarcoma, due to the presence of multifocally scattered large bizarre and often multinucleate cells with atypical hyperchromatic nuclei as well as mitotic figures which may be abnormal. Again, it is important to pay attention to the otherwise characteristic cytologic polymorphism of usual FH, entrapment of hyaline collagen bundles and overlying epidermal hyperplasia, as well as the clinical context which usually is that of an adult lower limb.

**Lipoma** showing extensive microscopic areas of fat necrosis is very frequently mistaken for atypical lipomatous tumor. The fat necrosis often results in marked variation in adipocyte size and the presence of histiocytes and multinucleate giant cells (some of which may be degenerate) arranged around liquefied adipocytes seems often to be mistaken for malignancy – however, such lesions entirely lack true adipocytic or stromal nuclear atypia or hyperchromasia.

**Spindle cell lipoma** quite often has a very prominent myxoid stroma and, particularly if adipocytes are few in number, then such myxoid examples can be mistaken for a variety of other tumor types. Most important among these is probably myxoid liposarcoma, since some examples of myxoid spindle cell lipoma have prominent thin-walled branching blood vessels. However, myxoid liposarcoma is extremely uncommon in the head and neck or upper back/shoulder region. Furthermore, spindle cell lipoma can be recognized by its distinctive uniform short stubby nuclei, often delicate cytoplasmic processes and ropey/refractile collagen bundles.

**Hibernoma**, when arising in the lower limb (particularly the thigh) is commonly dominated by multivacuolated lipoblast-like cells and more granular eosinophilic brown fat cells may be few in number and harder to recognize in this context – as a consequence, such lesions are often overinterpreted as atypical lipomatous/well-differentiated liposarcoma. Importantly,
however, the lipoblast-like cells usually have small central nuclei with evenly distributed chromatin, rather than the scalloped hyperchromatic nuclei of ALT.

**Lipogranulomas**, due to the introduction of exogenous lipids, most often for cosmetic purposes, seem to be increasing in frequency, given the modern obsession with physical ‘perfection’! Such lesions are easily mistaken for liposarcoma, particularly since the patient very often denies the introduction of lipid at that site. The facts that so many of the cells resemble multivacuolated lipoblasts (but lack hyperchromatic nuclei) combined with the almost invariable presence of admixed multinucleate giant cells are important diagnostic pointers.

**Plantar fibromatosis** is almost invariably hypercellular – and certainly substantially more cellular than its palmar counterpart. This hypercellularity is often mistaken for malignancy but the absence of atypia, the uniform fibroblastic/myofibroblastic morphology and the distinctively multinodular growth pattern within tendo-aponeurotic fibrous tissue are the principal clues to a benign diagnosis.

**Granular cell tumor** is only very rarely malignant and most examples of malignant granular cell tumor are large, deep-seated and show necrosis as well as significant atypia. Conversely, cutaneous granular cell tumors of ‘ordinary’ type may quite often show readily identified mitotic figures as well as mild nuclear atypia, possibly degenerative in type. In the context of a small cutaneous neoplasm showing no other atypical features, then the presence of such mitoses and nuclear alterations can safely be ignored.

**MALIGNANT LESIONS EASILY MISTAKEN AS BENIGN**

**Low-grade fibromyxoid sarcoma** is perhaps the best-known example of this potential pitfall, although these tumors are becoming more widely and reproducibly recognized since they were found to have a characteristic (7;16) translocation which can easily be detected by FISH or RT-PCR. These sarcomas usually have very bland cytology, typically with a swirling growth pattern and sharply alternating fibrous and myxoid areas. Within the myxoid areas, there are frequently arcades of thin-walled blood vessels. At least 50% of these tumors are EMA positive which can easily lead to a mistaken diagnosis of soft tissue perineurioma, in which context, molecular analysis may be invaluable. Despite the very bland appearance of LGFMS, these tumors have a significant rate of distant metastasis, often after a prolonged time interval, and therefore accurate diagnosis is important.
Malignant examples of solitary fibrous tumor are often deceptively uniform in cytologic terms, since frequently they lack any significant atypia or pleomorphism. In fact, many examples show only increased cellularity in areas of the tumor, sometimes quite subtle in degree. Since the most important criterion for recognizing SFT as malignant is the identification of more than 4 mitoses per 10 HPF, then a careful mitotic count in cases of SFT, particularly if there is any evident hypercellularity, is always worthwhile. In passing, it is worth remembering that occasional examples of otherwise entirely bland and pauci-mitotic SFT may give rise to distant metastasis but this is completely unpredictable on morphologic grounds.

Low-grade myxofibrosarcoma, which usually lacks metastatic potential but which often progresses to a higher-grade lesion in any recurrence, may be easily mistaken for a benign myxoid neoplasm such as cellular myxoma. The key distinguishing feature is the presence of nuclear atypia and hyperchromasia, not generally seen in any types of benign myxomatous lesion.

MISLEADING IMMUNOHISTOCHEMICAL STAINS

Perhaps the single best example of a misleading immunostain in the setting of soft tissue neoplasia is the increasingly indiscriminate use of MIB-1 to try and determine the biologic potential of a given lesion, despite the fact that there are very limited or (more often) no reproducible peer-reviewed data to show that such staining has any predictive value in the vast majority of types of soft tissue tumor. This practice, however, cannot be labeled as a ‘pitfall’ – instead, this is simply self-inflicted foolishness!

CD34 is widely regarded as a reliable discriminant between DFSP (almost invariably positive) and cellular fibrous histiocytoma. This is, however, a mistaken belief since as many as 5% of fibrous histiocytomas, particularly the cellular variant, may show quite convincing CD34 immunopositivity. Furthermore, CD34 staining in normal non-neoplastic dermal fibroblasts adjacent to an intradermal spindle cell proliferation is often misinterpreted. In truth, H&E morphology is by far the most reliable discriminant between DFSP and cellular FH.

CD117 positivity quite often leads to desmoid fibromatosis being misinterpreted as a gastrointestinal stromal tumor (GIST). However, unless antigen retrieval is used, only a very small minority of desmoid tumors are CD117 positive. Furthermore, there are clear-cut morphologic differences between these two tumor types – in particular, the long fascicular growth pattern and collagenous stroma which characterize desmoid tumors are essentially never seen in GISTs.
**CD99** immunopositivity in a poorly differentiated or round cell neoplasm is often utilized as evidence to favor a diagnosis of Ewing sarcoma/PNET. However, a very wide variety of different soft tissue tumors may show CD99 positivity, most often cytoplasmic or focal in distribution. By contrast, Ewing sarcoma/PNET characteristically shows CD99 positivity in perhaps 80-100% of tumor cells and the staining is distinctively membranous in distribution. Cytoplasmic positivity for CD99, whether focal or diffuse, is generally non-specific and of no significance.

**Spindle cell squamous cell carcinoma**, usually arising in either sun-damaged skin or else the upper aerodigestive tract, often goes underrecognized or misdiagnosed as either atypical fibroxanthoma or else some type of sarcoma because not enough keratin stains are performed. Although the majority of examples of spindle cell SCC express high molecular weight keratins (readily detected by pan-keratin antibodies such as MNF116), a subset of cases express only lower molecular weight keratins and it is therefore advisable to stain all such lesions for pan-keratin, AE1/AE3 and CAM 5.2 in order to enhance the likelihood of accurate diagnosis. p63 is often also helpful in this regard.

**Metastatic malignant melanoma**, as is well known, may show very substantial morphologic heterogeneity and may come to resemble almost any other tumor type. It is important to remember that few tumors are so consistently strongly and diffusely positive for S-100 protein in a high proportion of the neoplastic cells and therefore, when dealing with almost any otherwise non-distinctive malignant neoplasm showing strong S-100 positivity, melanoma should be at the top of the list of differential diagnostic possibilities. MPNST, other than when very neurofibroma-like, rarely shows such extensive S-100 protein staining. It is also important to remember that the large majority of poorly differentiated/pleomorphic and usually amelanotic metastases from melanoma will most often be entirely negative for second-line melanoma antigens. Conversely, it is also worth being aware that a small but significant subset of melanoma metastases may be immunonegative for S-100 protein but, at least in my experience, such lesions may quite often show misleading positivity for desmin (in the absence of other myogenic antigens). In a patient with an established history of malignant melanoma, this immunophenotype should not overrule a diagnosis of metastatic melanoma.

**Alveolar rhabdomyosarcoma** has been recognized in recent years to very often co-express synaptophysin and/or chromogranin. In the context of a round cell malignant
neoplasm, this can very easily give rise to confusion with a neuroendocrine carcinoma and, in my experience, this is most often a problem in tumors arising in the upper aerodigestive tract, particularly in adult patients.

**Solitary fibrous tumor** may be immunopositive for EMA in as many as 30% of cases and this can give rise to a mistaken diagnosis of synovial sarcoma. However, the patternless architecture and varying cellularity are quite different from the usual fascicular architecture of a synovial sarcoma. Furthermore, strong and diffuse positivity for CD34 is infrequent in synovial sarcomas.

**CONCLUSION**

By necessity, the examples listed above represent only a selected subset of the potential pitfalls in diagnosing soft tissue tumors. There are many additional pitfalls that arise, for example, through problems of sampling error, particularly in the era of tiny CT-guided needle biopsies. There are also a variety of pitfalls that relate specifically to histologic grading, a parameter regarded as being of the highest importance by many oncologists but which, in many cases, is determined entirely by the morphologic diagnosis. Unfortunately, it is not possible to cover the full range of diagnostic pitfalls in a brief presentation such as this.
Society for Ultrastructural Pathology
Cytogenetic Diagnosis of Soft Tissue Tumors: Avoiding Pitfalls

Julia A. Bridge, M.D. FACMG
University of Nebraska Medical Center

Overview

Conventional Cytogenetics

Molecular Cytogenetics

Conventional Cytogenetic Analysis

<table>
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<tr>
<th>Advantages</th>
<th>Diagnostically useful</th>
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<td>Provides global information in a single assay</td>
<td>Sensitive and specific</td>
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<td>• Includes primary and secondary anomalies</td>
<td>• Can be performed on fine-needle aspirates</td>
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<td>• Knowledge of anticipated anomaly or histologic diagnosis not necessary</td>
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<td>Variants undetectable by interphase FISH or RT-PCR may be uncovered</td>
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<td>Provides direction for molecular studies of pathogenetically important genes</td>
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Fresh Tissue Required

• Don’t fix tissue in formalin or freeze!

Avoid Pitfalls based on Tissue Submission Myths

• size
• sterility
• transportation
• turn-around-time

Dividing Cells are Required

Metaphase
**Tissue Size**

- 1 - 2 cm³ (0.5 - 1.0 g)
- FNA (also acceptable)

**Tissue Sterility**

- Don’t avoid submitting a specimen because it is not sterile!
- Discard non-neoplastic & necrotic tissues
- Pretreatment antibiotic/antifungal rinses prior to establishing culture
- Media also enhanced with antibiotics

**Tissue Transportation**

Tumor specimens can be successfully cultured after traveling long distances which may require 24-48 hours for delivery.

**Don’t avoid because you think it will take too long**

**24 Hour Turn-Around-Time**

- Obtain with direct harvest where endemic dividing cells are arrested after a 1-12 hour incubation in colchicine and culture medium

May be limited by in-vivo mitotic index.
**If in doubt, submit tissue**

- Studies can be cancelled the following day if it does not appear that cytogenetic studies will contribute to the diagnostic/prognostic process.

**61 year old female**

Myxofibrosarcoma (1998)

- 2007 - MRI suggested recurrence; incisional bx led to hematoma that has been non-responsive to serial evacuations and debridements.
- 2009 - 10 x 4 cm soft tissue mass right pelvis; no large fluid collection to suggest abscess or hematoma.

“Fibrous tissue with chronic inflammation, granulation tissue with recent and old hemorrhage”

47,XX,t(1;10)(p34;q22),del(3)(q12q25),del(5)(q13q35),der(7)add(p15)add(q32),del(11)(q13q23),del(14)(q13q24),del(15)(q22q24),add(17)(p13),inv(20)(p11.2p13),+r[20]
Issues

- Histopathologically, the lesion was initially missed – pt had undergone multiple evacuations/debridements with extensive recent and old hemorrhage and granulation tissue
- Re-review – rare, small foci of recurrent tumor (1-2 of >30 slides)
- Cytogenetics is sensitive, particularly if tumor cells have a proliferative advantage in culture

Avoid False Negative Results

Tempting to only analyze morphologically attractive metaphase cells

Avoid This Pitfall

Don’t interpret cytogenetic findings independent of the clinicohistopathologic findings

Often only the metaphase cells with poor chromosomal morphology are abnormal – don’t overlook these
35 year-old female

- MRI w and w/o contrast: solid/cystic mass (20 cm), posterior thigh
- Histologically: myxoid liposarcoma

Clear Cell Sarcoma

Oncogene 1996 Feb 1;12(3):489-94
Fusion of the EWS and CHOP genes in myxoid liposarcoma  Panagopoulos I, et al.

Myxoid/Round Cell Liposarcoma

Oncogene 1996 Feb 1;12(3):489-94
Fusion of the EWS and CHOP genes in myxoid liposarcoma  Panagopoulos I, et al.

In early 1996, ...

\[ t(12;22)(q13;q12) \]
Comparative Scale of Mapping

- Pitfall – forgetting the resolution of your method of analysis


Each chromosomal band has about 5 million base pairs of DNA (~50 genes)

- Chromosome 1 spans ~247 million bp and represents ~8% of total DNA
- Chromosome 1 contains >3,000 genes

57 year-old male

- Subcutaneous soft tissue mass arising on the back

Dermatofibrosarcoma Protuberans

- der(22)t(17;22)(q21;q13)

Ordinary, solitary lipoma

- t(17;22)(q21-22;q13)

PDGFβ

COL1A1-PDGFβ dual fusion probe set

Normal 17 homologues

Normal 22 homologues
**Beware of cryptic rearrangements**

- Metaphase cytogenetics has genomic resolution limited to defects that produce visible changes in chromosome number or banding pattern

**36-year-old male**

- Myxoid/round cell liposarcoma of his left calf

**der(16)t(1;16)(q11;q11)**

- Insertion of chromosome 12 material within a chromosome 22 homologue

**FISH with custom-designed PDGFβ break apart probe set**

**der(16) whole Chromosome 12 paint probe**

- Whole Chromosome 12 Paint Probe

- Insertion of chromosome 12 material within a chromosome 22 homologue
**Molecular Cytogenetic Analysis**

<table>
<thead>
<tr>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Can be performed on metaphase or interphase cell preparations (fresh, frozen or paraffin-embedded material)</td>
</tr>
<tr>
<td>Diagnostically useful</td>
</tr>
<tr>
<td>• Sensitive and specific</td>
</tr>
<tr>
<td>Prognostically useful</td>
</tr>
</tbody>
</table>

**Specimen Pitfalls**

- Fixative
- Truncation of nuclei
- Review tissue on the slide

**Avoid Prolonged Formalin Fixation or Decalcification Solution**

- Prolonged formalin fixation can produce a highly crosslinked (protein-protein and protein-nucleic acid) network or barrier adversely affecting the entry or penetration of FISH probes
- 5% Formic Acid (less than 24 hours) in Distilled Water or EDTA may be used

**Cytologic Touch Preparation or Tissue Scrape**

- Limited quantity of tissue
- Prior to decalcification
- Whole cell analysis
Slide Review Imperative

- Cells of interest must be identified on H & E to directly correspond to FISH analysis
- Almost exclusively scar or granulation tissue: a small focus of persistent or recurrent synovial sarcoma following a resection months earlier

Interpreting probe signal patterns in interphase FISH can be challenging and requires practice – certain pitfalls however, can be avoided…

- Ex: evaluation for amplification of an oncogene or loss of a tumor-suppressor gene locus should not only include the unique-sequence probe for that oncogene or tumor-suppressor gene locus, but also a second probe as a control for ploidy status
- Omission of a ploidy control could result in a false interpretation

- Amplified MYCN is associated with a poor prognosis regardless of clinical stage
- Patients with amplified MYCN are usually assigned to more intense therapeutic regimens

<table>
<thead>
<tr>
<th>6-12 copies of MYCN (consistent with amplification?)</th>
<th>Relative Duplication of MYCN (CEP2-MYCN ratio ~2)</th>
<th>Relative Duplication of MYCN (ratio ~2)</th>
<th>MYCN Amplification (ratio &gt;2)</th>
</tr>
</thead>
</table>

FISH for MYCN Amplification
**Pitfall – False Negative Break Apart Probes**

10 year-old female
* 8 cm soft tissue mass, left leg
* diagnosis of alveolar rhabdomyosarcoma favored

**FOXO1 (FKHR; 13q14) Break Apart Probe Set**

- Negative for rearrangement of FOXO1 locus; no split signals
- Increased copy number of FOXO1 locus (5 copies of fused red/green signals)

**Custom-Designed PAX3 (2q35) Break Apart Probe Set**

- Abnormal – three separate green signals (PAX3 distal) and three fused signals (“normal” PAX3 locus)

**Custom-Designed PAX3-FOXO1 Dual Fusion Probe Set**

- Positive for PAX3-FOXO1 fusions
- Three PAX3 signals
- Five FOXO1 signals

**Three Color FISH**

*PAX3 Distal FOXO1 Proximal FOXO1 Distal*

Abnormal
* PAX3 inserted between proximal and distal probes flanking FOXO1

**Cryptic Rearrangements**

Insertion of PAX3 within FOXO1 locus
Issues

- Histopathologically, the lesion was compatible with alveolar rhabdomyosarcoma
- Commercial FOXO1 break apart probe interpreted as negative for rearrangement (false negative)
- Availability of multiple probe sets is advantageous

36-year-old female

- 2-3 year history of painless, posterior right thigh mass
- Histopathologic diagnosis of low grade fibromyxoid sarcoma favored

Low Grade Fibromyxoid Sarcoma

- Supernumerary ring chromosome composed of chromosomes 7 and 16 or t(7;16)(q33;p11)
- FUS-CREB3L2 fusion

Break Apart FUS FISH

complex 1;7;16 translocation.
CREB3L1 (7q33)  
FUS (16p11.2)

? Break occurring centromeric to FUS
OR map not perfectly accurate and centromeric probe is slightly overlapping

Issues and Comments
- Ring chromosome composition was unexpected followed by misleading FUS break apart probe results
- Use of more than one probe set is helpful in clarifying results
Cytogenetic Diagnosis of Soft Tissue Tumors: Avoiding Pitfalls

Julia A. Bridge, M.D., FACMG

Society for Ultrastructural Pathology
2010
The evolution of mesenchymal tumor classification schemes has coincided with cytogenetic and molecular advances. Increasing recognition of the specific genetic abnormalities inherent in these tumors and the growing use of cytogenetic and molecular genetic procedures have aided formulation of a diagnosis and the resolution of cellular origin. A number of the genetic markers are also of prognostic value, and the importance of molecular testing for guiding targeted therapeutic strategies in mesenchymal neoplasia is emerging.

The following Tables list some of the pitfalls that may be encountered in the process of analyzing soft tissue tumors by conventional cytogenetic or molecular cytogenetic means and approaches for avoiding these pitfalls are recommended.

**Table 1: Potential Pitfalls for Conventional Cytogenetic Analysis**

<table>
<thead>
<tr>
<th>Tissue Submission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue must be fresh; it cannot be fixed or frozen because dividing cells are required for analysis.</td>
</tr>
<tr>
<td>1-2 cm³ is preferred, however, analysis can be conducted on much smaller specimens including fine needle aspirations</td>
</tr>
<tr>
<td>Sterile tissue is not required</td>
</tr>
<tr>
<td>Immediate transportation to the cytogenetic laboratory following surgical removal is ideal, however, successful analysis can occur 24-72 hours later</td>
</tr>
<tr>
<td>If in doubt, submit tissue, analysis can be cancelled the following day and only a nominal charge for setting up the culture is applied</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metaphase Cell Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avoid false negative results by including examination of metaphase cells with poor morphology (often only the metaphase cells with less optimal morphology are karyotypically abnormal and representative of the tumor)</td>
</tr>
<tr>
<td>Harvest supernatant; this may be the only portion with successful results</td>
</tr>
<tr>
<td>Rapid turn-around-time (24 hours) can be achieved with direct cell arrest</td>
</tr>
</tbody>
</table>
**Interpretation**

Must view the cytogenetic results in light of the clinical, radiographic and histopathologic findings.

Remain cognizant of the resolution of cytogenetic analysis (each chromosomal band contains approximately 5 million bp; ~50 genes); different tumor types may share similar cytogenetic breakpoints but the underlying genes involved are distinct.

Other molecular approaches such as FISH may be necessary to reveal cryptic rearrangements or define complex karyotypic abnormalities.

Anticipate the possibility of new variant translocations; they continue to be defined.

<table>
<thead>
<tr>
<th><strong>Table 2: Potential Pitfalls for Molecular Cytogenetic Analysis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue Submission</strong></td>
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<tr>
<td>Avoid prolonged formalin fixation (can produce a highly crosslinked protein-protein network or barrier adversely affecting the entry or penetration of FISH probes)</td>
</tr>
<tr>
<td>Avoid decalcification solution if possible (although FISH may be successful if used 5% formic acid for less than 24 hrs or EDTA)</td>
</tr>
<tr>
<td>Cytologic touch preparations - useful for tissue of limited quantities and can perform prior to subjecting the specimen to decalcification solutions</td>
</tr>
<tr>
<td>Imperative to review the corresponding H &amp; E to confirm lesional tissue is present and also to identify which cells should be scored by FISH</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Interpretation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenging and requires practice</td>
</tr>
<tr>
<td>Establishment of cut-off values is crucial and it is important to do so on the different types of specimens analyzed (touch preps vs 4-5 μm tissue sections)</td>
</tr>
</tbody>
</table>
Avoid underestimating subtle numerical or structural changes (particularly loss) with whole cell analysis – can be achieved through nuclear extraction (50 micron thick tissue section) or via cytologic touch preparation

Use a copy number control probe (control for ploidy status)

Variant signal patterns may have more than one interpretation; for example, loss of a proximal or distal break apart probe signal could be interpreted as a rearrangement of that gene locus or a coincidental deletion involving the location of that probe signal

Recommend having more than one type of probe set on-hand for a given locus (break apart and spanning probe sets)

Overall, it is prudent to have available more than one genetic diagnostic modality, to be ready to confirm unexpected or discrepant results by two independent techniques.