Paediatric small round cell sarcomas – an update

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The past several decades have been marked by phenomenal improvement in our ability to diagnosis the small blue cell tumors of childhood, traditionally including rhabdomyosarcoma, Ewing sarcoma (including PNET), neuroblastoma, lymphoma/leukemia, small cell osteosarcoma, and mesenchymal chondrosarcoma. Newer entities have been added, including desmoplastic small round cell tumor, extrarenal rhabdoid tumor, and BRD/NUT carcinoma. To a large degree, our increased recognition of classical entities and description of newer ones has been driven by a switch from immunohistochemistry to genetics as an ancillary diagnostic technique. With these advances has come a corollary question: how do we deal with cases that have clinical and histological features of specific pathological entities but lack their characteristic genetic features?

Two major genetic tests - *FOXO1* and *EWSR1* FISH - have driven diagnosis of most pediatric round cell sarcomas in the past decade. This is to be expected, as their corresponding pathological entities, rhabdomyosarcoma and Ewing sarcoma (as well as a host of non-Ewing EWS fusion-associated sarcomas), comprise the majority of pediatric sarcomas. Therefore, I will review recent advances in pediatric sarcomas in the practical diagnostic context of what to consider when these tests are negative.

**Fusion-negative rhabdomyosarcoma**

Rhabdomyosarcoma, the most frequent pediatric sarcoma, comprises two major histological species, embryonal (ERMS) and alveolar (ARMS) and variants of these patterns such as botryoid RMS. Genetic features that are useful in the context of diagnosing ERMS continue to be sparse, although certain chromosomal duplications and deletions recur¹, and loss of heterozygosity of 11p is typical². On the other hand, it is now established that ERMS characteristically shows a variably heterogeneous myogenin expression pattern, usually contrasting with the typical diffuse strong myogenin expression of ARMS³.

Historically, histology has been the gold standard for diagnosing ARMS, beginning with the recognition of the classical septate pattern by Riopelle and Theriault⁴. The criteria for diagnosing ARMS were loosened by the recognition of the “solid variant”, which lacks fibrous septa but shows similar cytology⁵. Histological classification of pediatric RMS became globally standardized with publication of the International Pediatric Sarcoma Classification (IPSC) in 1995⁶. Histological diagnosis of ARMS has been a critical component of standardized therapy, as they cannot be treated on low risk protocols and show more aggressive behavior in high and intermediate risk groups⁷. Sadly, no major improvements in ARMS treatment have been achieved since development of standard chemotherapy in the 1970s⁸. Thus, classification of RMS remains been a major concern for pediatric oncologists.
In spite of our inability to improve the survival of ARMS, in the past 30 years we have learned an amazing amount about its biology. These discoveries have followed the initial description of the ARMS-associated t(2;13) translocation and its genetic counterpart, the PAX3-FOXO1 (formerly PAX3-FKHR) fusion, broadened by findings of an alternate t(1;13) and associated PAX7-FOXO1 fusion in another subset. However, 20-30% of ARMS have persistently failed to display a translocation, to the dismay of finicky oncologists. Thus, the big question of the past decade has been, “Does the behavior of ARMS reflect its histology or its genetics?”

A giant step forward came with Davicioni et al.’s work on gene expression arrays. In these landmark papers, RNA extracted from a series of RMS was hybridized with Affymetrix gene chips, and the resultant morass of expression data was compared using histology as one parameter and fusion status as another. The results clearly indicate that fusion-negative ARMS cannot be distinguished from ERMS, whereas PAX-fusion positive ARMS gene expression indicates a distinctive genetic signature. This finding is perhaps not surprising, since downstream gene expression is being driven by the promoter region dysregulation associated with PAX fusion proteins. The results were repeated in subsequent expression array studies, and data emerged indicating that fusion-negative ARMS behave similarly to ERMS but not fusion-positive ARMS. The conclusions that have been reached are: 1) fusion-negative ARMS are clinically and molecularly indistinguishable from ERMS, and 2) PAX-fusion positive ARMS show a distinctive molecular signature unlike that of other tumors. However, questions persist: What exactly are fusion-negative ARMS? Does the diagnosis of “ARMS” depend on fusion-status, histology, or both?

In recent studies, possible sources of fusion-negative ARMS have emerged. These include: 1) ARMS with alternate fusions not detected with standard genetic tests; 2) new histological entities with features similar to ARMS, 3) tumors with mixed ARMS and ERMS histology, and 4) ERMS that look like ARMS. Alternate fusions not detected by standard genetic assays have indeed been discovered in recent years. However, they comprise less than 1% in reports published to date and include fusions of PAX3 with AFX1, NCOA1, and NCOA2. ARMS with the latter fusions may biologically be more akin to ERMS, but more data are needed.

Sclerosing RMS, a new histological entity, resembles ARMS and so was initially considered a “microalveolar” variant. In Menzel et al.’s seminal descriptions, only 7 adult tumors were reported, but 13 pediatric cases were added in a review of IRSG material by Chiles et al. Of note, only one of five tested cases contained a PAX fusion in Chile et al.’s series, and the single case tested in Folpe et al.’s series was also negative. Besides the microalveolar pattern, which lacks “floating clusters” and rows of septal-adherent cells, these tumors also differ from classical ARMS by their extensive osteoid-like sclerosis and low myogenin expression.

Tumors with mixed ARMS and ERMS features have posed a diagnostic quandary since early descriptions. The IPSC solved this dilemma by defining all mixed ARMS-ERMS as ARMS, regardless of the relative proportion of elements. Problems arose from this solution, as subsequent Intergroup Rhabdomyosarcoma Study Group (IRSG) and Children’s Oncology Group (COG) studies showed a rise in fusion-negativity from ~20% to 45% of ARMS, and the overall percentage of ARMS among RMS cases rose from 25-30% to 41%. The COG has responded by reverting to earlier IRSG criteria requiring >50% ARMS for the diagnosis. However, many mixed ARMS are fusion-negative, regardless of percentages, and occasional mixed cases with a minority ARMS component are fusion-positive (unpublished data).

The major problem in over-diagnosing ARMS can be surmised from IPSC definitions that indicate ERMS may occur as dense cellular sheets, so that the difference from solid ARMS becomes a relatively subtle
cytological one. Solid ARMS contain round, monomorphous nuclei whereas dense ERMS contain nuclei with spindly or irregular profiles, and some overlap may exist. Ancillary testing is helpful in this distinction, as myogenin expression is generally strong and diffuse in ARMS and heterogeneous in ERMS\(^3\). Some overlap occurs in cases with 50-90% myogenin positivity, but expression array studies have produced new markers like AP2\(\beta\) that may be superior\(^20\).

To summarize recent RMS findings, it appears that ARMS can easily be over-diagnosed, which has contributed to studies indicating that fusion-negative ARMS have biological similarity to ERMS. The clinical significance of fusion-negative ARMS has not been thoroughly tested, as little data exists for ARMS treatment with low risk therapy. The likelihood of a lesion being fusion-negative is higher with solid variant ARMS than with classical ARMS\(^21\), but in the absence of molecular testing, myogenin staining can be helpful in making this diagnosis\(^3\). Stratification in future protocols will be based on genetic classification rather than histology, as the exact definition of ARMS has become a source of controversy\(^22,23\).

**Fusion-negative Ewing sarcoma**

Although fusion-negative Ewing sarcomas/PNETs have been reported\(^24,25\), their existence is also questioned. In some cases, there is a discrepancy between RT-PCR and FISH results, the latter appearing to be a more sensitive (but less specific) technique\(^24,26\). This may result from the wide array of \(EWS\) fusion partners that occur with Ewing sarcomas\(^25\). In fusion-negative lesions with Ewing histology and CD99-positivity, it behooves one to rule out other diagnoses, the most likely ones being rhabdomyosarcoma, neuroblastoma, and lymphoma. Rhabdomyosarcomas can be excluded with myogenin and desmin stains, with the caveat that rare PNETs are desmin-positive but show other features of neuroepithelial differentiation or genetic change\(^27\). Undifferentiated neuroblastomas lacking rosettes or neuropil should also be separated from Ewing sarcomas. Undifferentiated neuroblastomas are aggressive neoplasms, automatically unfavorable in Shimada grading\(^28\), but unlike Ewing sarcoma they show CD99-negativity and CD56-positivity\(^29,30\). Lymphomas can be particularly treacherous, as lymphoblastic tumors often show CD99-positivity and CD45-negativity, but CD3, CD43, and/or TdT are usually positive\(^31\).

**Undifferentiated sarcoma**

In current practice, fusion-negative Ewing-like neoplasms are most often diagnosed as undifferentiated sarcomas. In recent COG studies, undifferentiated sarcomas have been treated as non-rhabdomyosarcomatous soft tissue sarcomas, and preliminary results suggest that they respond to chemotherapy (unpublished data). \(FUS\) FISH testing is recommended, however, as a subset of Ewing sarcomas show alternate \(FUS-ERG\) or \(FUS-FEV\) fusions, and \(FUS\) and \(EWS\) are related genes\(^25\). A new fusion, \(CIC-DUX4\), derived from a t(4;19)(q35;13.1) translocation\(^32\), is found in a substantial number of undifferentiated sarcomas. Italiano et al.\(^33\) found \(CIC\) rearrangements in 15 of 22 \(EWS\) fusion-negative Ewing-like cases.

**BRD4-NUT carcinoma**

An undifferentiated, high-grade carcinoma that often affects younger patient and resembles Ewing tumors has been recently described and is named for its characteristic gene fusion, \(BRD4-NUT\). The fusion is formed by a t(15;19)(q13;q13) translocation\(^34\). Tumors of this type typically affect midline structures cervicothoracic structures such as the upper respiratory tract and mediastinum, although the list is widening\(^35\). They are highly aggressive tumors that pursue an almost invariable fatal course. NUT carcinomas resemble small cell carcinomas of adults and predominately comprise sheets of small
undifferentiated cells. Well-defined foci of squamous differentiation often abruptly appear but may be absent in limited biopsies. NUT carcinomas typically show strong cytokeratin differentiation, but cytokeratin may also be expressed by a minority of Ewing sarcomas. Diagnosis requires either genetic or immunohistochemical NUT confirmation, and other poorly differentiated pediatric carcinomas, including EBV-associated nasopharyngeal carcinoma, mucoepidermoid carcinoma, and germ cell tumors, should be excluded.

**Extrarenal rhabdoid tumor**

Rhabdoid tumors, known for their epithelial or rhabdomyosarcoma-like appearance, on occasion form sheets of lymphoma-like cells that can resemble Ewing sarcoma. The lymphomatoid pattern was first described by Weeks et al. in their description of the histological diversity of rhabdoid tumors of the kidney. In these lesions, characteristic hyaline inclusions may be inconspicuous or absent. A similar phenomenon occurs with proximal variants of epithelioid sarcomas, which exhibit identical genetic changes. These lesions are generally recognized today by INI1-negativity, which separates them from the strongly positive Ewing sarcomas. The list of INI1-negative neoplasms grows ever longer and now includes renal medullary carcinoma, extraskeletal myxoid chondrosarcoma, epithelioid MPNST, myoepithelial carcinoma, small cell hepatoblastoma, and poorly differentiated chordoma, none of which are often included in the differential diagnosis of Ewing sarcoma. A subset of synovial sarcomas shows reduced INI1 expression, and this diagnosis should always be considered with fusion-negative Ewing-like sarcomas.

**Synovial sarcoma**

In soft tissue pathology, synovial sarcoma is the great imitator. It occurs in a wide variety of locations and can exhibit features identical to Ewing sarcoma, including monomorphic round cell histology and CD99 positivity. Thus, it should always be considered in EWS fusion-negative sarcomas resembling Ewing sarcoma, even if cytokeratin and EMA stains are negative. An illustrative case with extensive bony invasion is presented, in which diagnosis was accomplished via FISH demonstration of SYT rearrangement (courtesy of Dr. Zhongxin Yu, University of Oklahoma). In the post-chemotherapy resection specimen, abundant glands were present.

**Summary**

Adoption of genetic techniques in diagnosis of pediatric sarcomas has improved recognition of many lesions but raised diagnostic quandaries in fusion-negative ones. Small cell sarcomas may resemble ARMS or Ewing sarcoma but lack characteristic fusions, creating diagnostic dilemmas. With fusion-negative ARMS, future protocols will rely on genetics rather than histology for stratification, although fusion-associated immunomarkers may come to be used as substitutes. For fusion-negative Ewing-like sarcomas, it is wise to do a full panel of diagnostic markers, including SYT FISH, to exclude other lesions. Undifferentiated pediatric soft tissue sarcomas lacking EWS fusions are treated as non-RMS soft tissue sarcomas; many contain a unique CIC-DUX4 fusion.

**References**


Sumegi, J. et al. Recurrent t(2;2) and t(2;8) translocations in rhabdomyosarcoma without the canonical PAX-FOXO1 fuse PAX3 to members of the nuclear receptor transcriptional coactivator family. *Genes, chromosomes & cancer* 49, 224-236, doi:10.1002/gcc.20731 (2010).


Paediatric small round cell sarcomas – an update

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Pediatric round cell sarcomas: classical types and differential diagnosis

- Rhabdomyosarcoma
- Ewing sarcoma (including PNET)
- Neuroblastoma
- Lymphoma/leukemia
- Small cell osteosarcoma
- Mesenchymal chondrosarcoma
- Undifferentiated sarcoma
Pediatric small cell sarcomas – newer entities in the differential diagnosis

- Desmoplastic small round cell tumor
- Extrarenal rhabdoid tumor
- BRD/NUT carcinoma
Diagnosis in the past decade: a shift from immunohistochemistry to genetics
What do I do when the genetic test for a small round cell tumor is negative?
Two major genetic tests for round cell sarcomas

- *FOXO1* FISH: alveolar rhabdomyosarcoma (ARMS)
- *EWSR1* FISH: Ewing sarcoma
Rhabdomyosarcoma: the most frequent pediatric soft tissue sarcoma

Sarcoma incidence per million (source: WHO)

- Rhabdomyosarcomas
- Non-rhabdos
Rhabdomyosarcoma: assignment of therapy

• Low risk
• Intermediate risk
• High risk
• Combination of stage (clinical), group (pathological), age, and histological subclassification
Histology: the historical gold standard for classification

Classical ARMS as described by Riopelle and Theriault
A 1980s change in paradigm: solid variant
Current criteria for classification: the International Pediatric Sarcoma Classification
Diagnosis of ARMS: a high stakes game

• Not treated on low risk protocols
• Worse outcome with high risk protocols
• May be aggressively treated with intermediate risk protocol.
• No truly significant improvements in therapy since 1970s (compared to embryonal RMS [ERMS]).
Outcome in ARMS vs ERMS

Metastatic RMS, IRSG study IV

Survival vs Time

ARMS

ERMS

p=0.028

favorable

unfavorable

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RMS diagnosis: MyoD and myogenin

- Transcription factors that bind DNA and initiate myogenesis
- Nuclear staining is highly sensitive and specific for rhabdomyogenesis
- Cytoplasmic staining is non-specific
Myogenin staining varies according to subtype

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ERMS

ARMS
Another change in paradigm: genetics
Fusion distribution in ARMS

- PAX3-FOXO1: 50%
- PAX7-FOXO1: 20%
- PAX fusion negative: 30%
The question of the past decade:
Does ARMS behavior reflect histology or genetics?
Identification of a PAX-FKHR Gene Expression Signature that Defines Molecular Classes and Determines the Prognosis of Alveolar Rhabdomyosarcomas

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Array studies: RMS gene expression clusters by fusion positivity, not by histology

Red: ARMS; Blue: ERMS; Green: fusion-negative; Yellow: fusion-positive
Survival correlates with fusion

• 5 year overall survival:
  – ERMS: 75%
  – Fusion negative ARMS: 75%
  – Fusion positive ARMS: 25%

• Metastatic rate:
  – ERMS: 15%
  – Fusion negative ARMS: 20%
  – Fusion positive ARMS: 45%
Conclusion

• Fusion-negative ARMS is clinically and molecularly indistinguishable from ERMS
• Molecular results confirmed in four independent international studies
• Question: does diagnosis of ARMS depend on fusion, histology, or both?
Possible sources of fusion-negative ARMS

• Alternate fusions not detected with standard tests
• New histological entities with similar features
• “Mixed ARMS-ERMS”: lesions with features of both ARMS and ERMS
• Embryonal rhabdomyosarcoma look-alikes
Alternate fusions not detected with standard tests

- **PAX3-AFX1**
- **PAX3-NCOA1**
- **PAX3-NCOA2**
- All involve forkhead type proteins
- All negative with FOXO1 FISH
- Rare; <1% of ARMS
New histopathological entity with similar features:
Sclerosing RMS

• Four cases of adult RMS characterized by the production of abundant, hyalinized osteoid, or chondroid-like matrix

• Initially considered probable variants of ARMS because of occurrence in extremities of adults, microalveolar pattern, and primitive round cell morphology
Pediatric sclerosing RMS:

- Thirteen patients had features of sclerosing RMS.
- 9 had been diagnosed as ARMS, 3 as ERMS, and 1 as spindle cell RMS.
- Only 1 of 5 tested cases had a PAX fusion demonstrated.
Mixed ARMS: tumors with features of both ERMS and ARMS
International Classification (1995)

• “Traditionally, these tumors have been called mixed alveolar/embryonal RMS or embryonal if the alveolar pattern was less prominent than the embryonal one.

• In the proposed classification, any alveolar features are sufficient to classify the tumor as alveolar RMS.”
Relative proportions of fusion-status vs IRSG study period

- **PAX3-FOXO1**
  - IRS3: [Bar Height]
  - IRS4: [Bar Height]
  - Post-IRS4: [Bar Height]

- **PAX7-FOXO1**
  - IRS3: [Bar Height]
  - IRS4: [Bar Height]
  - Post-IRS4: [Bar Height]

- **Fusion negative**
  - IRS3: [Bar Height]
  - IRS4: [Bar Height]
  - Post-IRS4: [Bar Height]
## Percentage of ARMS increase, COG report

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<tr>
<td><strong>Number of RMS</strong></td>
<td>2003</td>
<td>864</td>
<td>968</td>
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<tr>
<td><strong>Number of ARMS</strong></td>
<td>511</td>
<td>262</td>
<td>400</td>
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<tr>
<td><strong>Per cent</strong></td>
<td>25.5</td>
<td>30</td>
<td>41</td>
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Protocol adjustments

- IRS3: 50% ARMS required for diagnosis
- IRS4: ARMS diagnosis not used to stratify therapy.
- Post-IRS4: Any ARMS foci make diagnosis
ERMS that look like ARMS
Dense ERMS: The International Classification

• “The histologic pattern of embryonal RMS is predominantly that of a moderately cellular tumor with loose myxoid stroma, although some dense areas may occur frequently. “

• “Some tumors may consist exclusively of fields of closely packed cells. “
Solid ARMS: International Classification diagnosis

• “The ”solid” variant of alveolar RMS grows as solid masses of closely aggregated cells, with no or scarcely discernible alveolar arrangement.”

• “No marked compartmentalization and no distinct alveolar configuration is apparent.”
“Solid” variant ARMS
A very subtle distinction
Myogenin staining
Conclusions

• ARMS can easily be overdiagnosed.
  – Myogenin staining can be helpful.
• Solid variant “ARMS” are often fusion-negative.
• The clinical significance of fusion-negative ARMS remains to be determined, but current studies show no differences from ERMS
• Future COG stratification will be based on fusion status.
What is fusion-negative “Ewing sarcoma”?

- Rhabdomyosarcoma, neuroblastoma, and lymphoma should be excluded
- Undifferentiated sarcoma
- *BRD4-NUT* carcinoma
- Extrarenal rhabdoid tumor
- Synovial sarcoma
Ewing sarcoma histology

Classical Ewing sarcoma

Primitive neuroectodermal tumor
Ewing sarcoma genetics

- t(11;22)(q24;q12): EWSR1-FLI1 fusion
- t(21;22): EWSR1-ERG fusion
- Other EWSR1 fusion partners FEV, E1AF, ETV1
  - New non-ETS genes POU5F1, ZNF278, SP3
- Rare FUS-FEV or FUS-ERG fusions
  - Use FUS FISH probe in typical cases with negative EWSR1 fusion results
Undifferentiated sarcoma, round cell type

• Negative for all phenotypic markers
  – Focal neural positivity?
• Treated as non-rhabdo sarcoma by COG
• High prevalence of new fusion type: *CIC-DUX4*
  – t(4;19)(q35;13.1)
  – Memorial study: *CIC* rearrangements found in 15/22 *EWSR1* fusion-negative cases
  • CD99 diffuse positive, patchy positive, or negative
  • Mostly negative for other markers
BRD4-NUT carcinoma

• Predominately affects young patients, but wide age range
• Usually involves midline structures: upper respiratory tract, mediastinum
• Characterized by t(15;19)(q13;p13.1) translocation
• Almost invariably fatal course
BRD4-NUT carcinoma

- Resembles small cell carcinoma.
- May not show obvious epithelial differentiation on H and E stain, but should be cytokeratin-positive (as are 15% of Ewing sarcoma).
- Squamous differentiation is often abrupt.
Undifferentiated *BRD4-NUT* carcinoma

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Courtesy of Sara Vargas, MD, Boston Children’s Hospital
Differentiating *BRD4-NUT* carcinoma

Courtesy of Sara Vargas, MD, Boston Children’s Hospital
BRD4-NUT carcinoma: cytokeratin

Courtesy of Sara Vargas, MD, Boston Children’s Hospital
BRD4-NUT carcinoma NUT stain

Courtesy of Sara Vargas, MD, Boston Children’s Hospital
Also consider:

• EBV-associated carcinoma (nasopharyngeal carcinoma)
• Mucoepidermoid carcinoma
• Keratin-positive carcinomas, such as rhabdoid tumor
• Poorly differentiated germ cell tumor
Extrarenal rhabdoid tumor

- Wide variety of primary sites, including soft tissue and skin
- Primarily affects young children, with overlap with epithelioid sarcoma in older ones
- Diverse histologies, including “lymphomatoid” pattern described by Weeks and Beckwith.
Extrarenal rhabdoid tumor
INI1
Tumors with negative INI1

- Rhabdoid tumor
- Epithelioid sarcoma
- Renal medullary carcinoma
- Extraskeletal myxoid chondrosarcoma
- Epithelioid MPNST

- Myoepithelial carcinoma
- Small cell hepatoblastoma
- Poorly differentiated chordoma
- Reduced in synovial sarcomas
Final illustrative case
Courtesy of Zhongxin Yu, MD
University of Oklahoma

• 14 years old with left hip pathological fracture
CD99: negative
INI-1: negative (occasional positive)
Vimentin: negative
Other IHC

- Lymphoma markers: negative
  - (CD3, CD5, CD20, CD43, CD79a, PAX5, TdT, CD117, CD34)
- Myeloperoxidase: weakly positive, focal
- Myogenin and desmin: negative
- S-100: negative
- NSE and synaptophysin: negative
- CD68, CD163: negative
- EMA and pan-cytokeratin: negative
Preliminary Diagnosis

• Small round blue cell tumor of the bone
  – ? Ewing’s sarcoma
    • Pending EWS FISH result
  – ? Ewing’s – like small cell osteosarcoma
    • Pending FISH to rule out Ewing’s sarcoma
    • ?rebiopsy to get more tissue with osteoid
Final diagnosis

- FISH is negative for *EWSR1* rearrangement
- FISH is positive for *SYT(SS18)* rearrangement
- Final diagnosis—Synovial sarcoma
Resection specimen histology (post chemotherapy)
Summary

• Genetics improves diagnosis of many pediatric sarcomas but raises diagnostic quandaries in others.

• Lesions that resemble ARMS or Ewing sarcoma may lack fusions, creating diagnostic problems and delay.
Summary

• Future protocols will rely on genetics of RMS rather than histology for stratification
  – Fusion-associated immunomarkers may be used as substitutes.

• Fusion-negative Ewing-like sarcomas require a full panel of diagnostic markers, including SYT FISH.

• Undifferentiated pediatric sarcomas are treated as non-RMS soft tissue sarcomas
  – Many contain a unique CIC-DUX4 fusion.