Protocol for the Examination of Biopsy Specimens from Pediatric Patients with Rhabdomyosarcoma

Version: 5.0.0.1
Protocol Posting Date: June 2025
CAP Laboratory Accreditation Program Protocol Required Use Date: June 2024
The changes included in this current protocol version do not affect the prior accreditation date.

For accreditation purposes, this protocol should be used for the following procedures AND tumor types:

Procedure	Description
Biopsy	Includes specimens designated core biopsy, incisional biopsy, excisional biopsy, or other
Tumor Type	**Description**
Rhabdomyosarcoma	Includes pediatric patients with all rhabdomyosarcoma variants and ectomesenchymoma

The following should NOT be reported using this protocol:

Procedure
Resection (consider Rhabdomyosarcoma Resection protocol)
Tumor Type
Adult Rhabdomyosarcoma[#] (consider using soft tissue protocol)

[#]Rhabdomyosarcoma in adults may be treated differently than pediatric rhabdomyosarcoma and use of the AJCC TNM staging system remains appropriate for adult patients.

Version Contributors

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Glossary:

Author: Expert who is a current member of the Cancer Committee, or an expert designated by the chair of the Cancer Committee.
Expert Contributors: Includes members of other CAP committees or external subject matter experts who contribute to the current version of the protocol.

Accreditation Requirements

Synoptic reporting with core and conditional data elements for designated specimen types* is required for accreditation.

- Data elements designated as core must be reported.
- Data elements designated as conditional only need to be reported if applicable.
- Data elements designated as optional are identified with "+". Although not required for accreditation, they may be considered for reporting.

This protocol is not required for recurrent or metastatic tumors resected at a different time than the primary tumor. This protocol is also not required for pathology reviews performed at a second institution (i.e., second opinion and referrals to another institution).

Full accreditation requirements can be found on the CAP website under Accreditation Checklists.

A list of core and conditional data elements can be found in the Summary of Required Elements under Resources on the CAP Cancer Protocols website.

**Includes definitive primary cancer resection and pediatric biopsy tumor types.*

Synoptic Reporting

All core and conditionally required data elements outlined on the surgical case summary from this cancer protocol must be displayed in synoptic report format. Synoptic format is defined as:

- Data element: followed by its answer (response), outline format without the paired Data element: Response format is NOT considered synoptic.
- The data element should be represented in the report as it is listed in the case summary. The response for any data element may be modified from those listed in the case summary, including "Cannot be determined" if appropriate.
- Each diagnostic parameter pair (Data element: Response) is listed on a separate line or in a tabular format to achieve visual separation. The following exceptions are allowed to be listed on one line:
    - Anatomic site or specimen, laterality, and procedure
    - Pathologic Stage Classification (pTNM) elements
    - Negative margins, as long as all negative margins are specifically enumerated where applicable
- The synoptic portion of the report can appear in the diagnosis section of the pathology report, at the end of the report or in a separate section, but all Data element: Responses must be listed together in one location
- Organizations and pathologists may choose to list the required elements in any order, use additional methods in order to enhance or achieve visual separation, or add optional items within the synoptic report. The report may have required elements in a summary format elsewhere in the report IN ADDITION TO but not as replacement for the synoptic report i.e., all required elements must be in the synoptic portion of the report in the format defined above.

Summary of Changes

v 5.0.0.1

- Accreditation statement update
- eCP explanatory note electronic link updates

Reporting Template

Protocol Posting Date: June 2025
Select a single response unless otherwise indicated.
CASE SUMMARY: (RHABDOMYOSARCOMA AND RELATED NEOPLASMS: Biopsy)

EXPERT CONSULTATION

+Expert Consultation (Note A)
___ Pending - Completion of this CAP Cancer Protocol is awaiting expert consultation
___ Completed - This CAP Cancer Protocol or some elements have been performed following expert consultation
___ Not applicable

SPECIMEN (Note B)

Procedure (Note C)
___ Core needle biopsy
___ Incisional biopsy
___ Excisional biopsy
___ Other (specify): _____
___ Not specified

TUMOR

Tumor Site
___ Bile duct
___ Bladder / prostate
___ Cranial / parameningeal
___ Extremity
___ Genitourinary (excluding bladder / prostate)
___ Head and neck (excluding parameningeal)
___ Orbit
___ Other(s) (includes trunk, retroperitoneum, etc.) (specify): _____
___ Not specified

Tumor Size (required only for excisional biopsy)
___ Not applicable
___ Greatest dimension in Centimeters (cm): _____ cm
+Additional Dimension in Centimeters (cm): ____ x ____ cm
___ Cannot be determined (explain): _____

Histologic Type (Note D)
___ Embryonal
___ Alveolar
___ Spindle cell / sclerosing
___ Ectomesenchymoma

___ Rhabdomyosarcoma, not otherwise specified (NOS): _____
___ Other (specify): _____
+Histologic Type Comment: _____

Anaplasia (Note E)
___ Not identified
___ Focal (single or few scattered anaplastic cells)
___ Diffuse (clusters or sheets of anaplastic cells)
___ Cannot be determined: _____

+Tumor Comment: _____

MARGINS (Note F)

Margin Status (required only for excisional biopsy)
___ Not applicable
___ All margins negative for tumor
Distance from Tumor to Closest Margin
Specify in Centimeters (cm)
___ Exact distance: _____ cm
___ Greater than: _____ cm
___ At least: _____ cm
___ Less than: _____ cm
___ Less than 1 cm
___ Other (specify): _____
___ Cannot be determined: _____
Closest Margin to Tumor
___ Specify closest margin(s): _____
___ Cannot be determined (explain): _____
___ Tumor present at margin
Margin(s) Involved by Tumor
___ Specify involved margin(s): _____
___ Cannot be determined (explain): _____
___ Other (specify): _____
___ Cannot be determined (explain): _____

+Margin Comment: _____

ADDITIONAL FINDINGS

+Additional Findings (specify) (Note G): _____

SPECIAL STUDIES

Gene Fusion Studies (Note H)
___ Not performed

___ Results pending
___ No FOXO1 rearrangement
___ FOXO1 rearrangement present
  ___ Fusion partner not known
  ___ FOXO1-PAX3 gene rearrangement
  ___ FOXO1-PAX7 gene rearrangement
___ Other (e.g., PAX3-NCOA1 or other variant translocation) (specify): _____

Method for Gene Fusion Studies

  ___ Not applicable (Gene Fusion Studies not performed)
  ___ Conventional karyotyping
  ___ Fluorescent in situ hybridization (FISH)
  ___ Reverse transcriptase polymerase chain reaction (RT-PCR)
  ___ Sequencing (specify type, if known): _____
  ___ Other (specify): _____
  ___ Not known

+Molecular Genetic Studies (Note I) (select all that apply)

___ Not performed
___ Pending
___ DICER1 mutation
___ MyoD1 L122R point mutation
___ VGLL2 / NCOA2 gene fusions (specify): _____
___ EWSR1 / FUS-TFCP2 gene fusion (specify): _____
___ Other (specify): _____

+Method for Molecular Genetic Studies (select all that apply)

  ___ Fluorescent in situ hybridization (FISH)
  ___ Reverse transcriptase polymerase chain reaction (RT-PCR)
  ___ Sequencing (specify type, if known): _____
  ___ Other (specify): _____

COMMENTS

Comment(s): _____

Explanatory Notes

A. Expert Consultation

Expert consultation is not required. This question has been added to annotate, if so desired, that the case has been sent out for consultation and thus items of the CAP protocol could not be completed pending expert consultation. Completion of the CAP protocol will then be performed following consultation.

B. Submission of Tissue

If feasible, a minimum of 100 mg of viable tumor should be snap-frozen for potential molecular studies.[1] If tissue is limited, the pathologist can keep the frozen tissue aliquot used for frozen section (usually done to determine sample adequacy and viability) in a frozen state (-80°C or lower), with the proviso that routine examination of this tissue may be required if the tissue is otherwise inadequate. Molecular studies to evaluate fusion status, FISH or RT-PCR, may be performed on paraffin sections or frozen tissue. When material is scant, FISH can also be performed on touch preparations made from fresh material obtained at the time of biopsy.

References

1. Qualman SJ, Morotti RA. Risk assignment in pediatric soft-tissue sarcoma: an evolving molecular classification. *Curr Oncol Rep.* 2002; 4:123-130.

C. Procedures

Core needle biopsies can obtain sufficient material for special studies and morphologic diagnosis, but sampling problems may limit tumor subtyping. Inadequate sampling with needle biopsies may be related to specimen size, necrosis, hemorrhage, crush artifact, and specimen adequacy.[1] Open incisional biopsy consistently provides a larger sample of tissue and maximizes the opportunity for a specific pathologic diagnosis.[2] Excisional biopsy may not include an adequate margin of normal tissue, even with an operative impression of total gross removal.[2] For all types of resections, marking (inking followed by use of a mordant) and orientation of the specimen (prior to cutting) are mandatory for accurate pathologic evaluation.[2]

References

1. Willman JH, White K, and Coffin CM. Pediatric core needle biopsy: strengths and limitations in evaluation of masses. *Pediatr Dev Pathol.* 2001;4(1):46-52.
2. Coffin CM, Dehner LP. Pathologic evaluation of pediatric soft tissue tumors. *Am J Clin Pathol.* 1998;109(suppl 1): S38-S52.

D. Histologic Type

The International Classification of Rhabdomyosarcoma classified childhood rhabdomyosarcoma (RMS) into prognostically useful histologic categories.[1] However, studies show that *FOXO1* fusion status drives unfavorable outcome for children with rhabdomyosarcoma, and histologic classification is no longer the primary tool for determining prognosis and risk stratification.[2,3] That notwithstanding, a consistent and appropriate designation of histologic subtype remains important due to its universal applications. The 5th edition of *WHO Classification of Tumours of Soft Tissue and Bone* defines the histologic classification of rhabdomyosarcoma in 4 categories: embryonal (including botryoid), alveolar, spindle cell/sclerosing, and pleomorphic subtypes.[4] Pleomorphic RMS is exceedingly rare and not well characterized in the pediatric population; many of these cases can be considered RMS with diffuse anaplasia. In addition to these

subtypes, recent studies have further characterized the clinicopathologic and molecular subtypes of spindle cell rhabdomyosarcoma; however, to date, all biologic subtypes still fall within the histologic category of spindle cell RMS in the WHO Classification.[4] This pattern, as well as ectomesenchymoma (RMS with ganglion cell or neuroblastic differentiation) and other histologic patterns are discussed in more detail below. Finally, RMS, not otherwise specified (NOS), is reserved for cases where there is insufficient material for confident histologic classification.

Embryonal Rhabdomyosarcoma

Embryonal RMS (ERMS) includes the typical, dense and botryoid patterns of RMS. These patterns account for over one-half of all RMS. Embryonal RMS is composed of mesenchymal cells that show variable degrees of cytoplasmic skeletal muscle differentiation. They are moderately cellular, but in the typical pattern often contain both hypo- and hypercellular areas with a loose, myxoid stroma. Either of these components may predominate, particularly in limited biopsies. Sampling of uniformly hypercellular regions produces a dense pattern of embryonal RMS that may resemble solid alveolar RMS. The typical immunohistochemical staining pattern of ERMS, with myogenin (myf4) staining most often seen in less than half of embryonal RMS nuclei, absent AP2 beta staining and strong diffuse expression of HMGA2 support this diagnosis.[5] Testing for *PAX-FOXO1* translocations may also assist in making this distinction.[6]

In embryonal RMS, tumor cells may be rounded, stellate, or spindle-shaped. Nuclei are generally small with a light chromatin pattern and inconspicuous nucleoli, although occasionally large central nucleoli may be seen. They typically have more irregular or spindled outlines than those of alveolar RMS. Many tumor cells contain generous amounts of eosinophilic cytoplasm, a feature of rhabdomyoblastic differentiation. Cells with elongated tails of cytoplasm (“tadpole cells”) and cells with cytoplasm in the shape of a ribbon or “strap” are helpful in the light-microscopic diagnosis. Cross-striations can be seen in less than one-half of the cases and are not a prerequisite for diagnosis. The dense pattern of embryonal RMS shows similar cytologic features, although rhabdomyoblastic differentiation is minimal.[6] Adjacent to an epithelial surface, embryonal RMS shows a botryoid pattern, particularly in the bladder, vagina, nasal cavity and sinuses, and biliary tract. These botryoid variants demonstrate a cambium layer (condensed layer of rhabdomyoblasts) underlying an intact epithelium. A subset of embryonal RMS are associated with either sporadic or germline *DICER1* mutations.[7] These *DICER1* mutated embryonal RMS are most commonly located in the uterine corpus or uterine cervix,[7,8] although they are also described less frequently in other locations including the ovary, fallopian tube, or intracranial sites.[9,10] Histologically, *DICER1* mutated embryonal RMS often contain heterologous elements to include nodules of cartilage, osteoid, or other non-rhabdomyomatous components; these tumors are often histologically similar to that seen in pleuropulmonary blastoma (PPB).[7,8,9,10] Rarely, embryonal RMS may be predominantly epithelioid (or rhabdoid-like).[11]

The differential diagnosis of embryonal RMS includes the sclerosing and spindle cell variants of RMS, as well as the solid pattern of alveolar RMS. Embryonal RMS is often quite heterogeneous, and small foci of a spindled or sclerosing pattern are commonly seen, particularly in primary resections of large paratesticular or retroperitoneal masses. A dominant (at least 80%) spindled or sclerosing pattern is required for diagnosis of this RMS subtype. Ectomesenchymoma (discussed below) typically has embryonal RMS along with a neuroblastic or ganglion cell component. Undifferentiated embryonal sarcoma of the liver has some morphologic and phenotypic overlap, but it generally does not express MYOD1 (myf3) or myogenin by immunohistochemistry and contains characteristic cytoplasmic hyaline globules. Embryonal RMS-like differentiation is a common component of the multipatterned pediatric lung

tumor pleuropulmonary blastoma. Occasional Wilms tumors show marked skeletal muscle differentiation, particularly after chemotherapy, and may even have a cambium layer in tumors abutting the renal pelvis. Well-differentiated embryonal RMS can also have some morphologic overlap with fetal rhabdomyoma. The finding of increased mitoses (>15 per 50 high-power fields), marked hypercellularity, a "cambium layer," and atypical nuclear features are more characteristic of RMS. Giant cell tumors of tendon sheath may lack giant cells, contain cells with eosinophilic cytoplasm, and show desmin positivity; however, they are strongly CD68 positive and myogenin negative. Pseudosarcomatous fibroepithelial polyps of the lower female genital tract are particularly treacherous and should be considered in botryoid lesions occurring in adolescents and adults, particularly during pregnancy. These hypercellular lesions contain pleomorphic cells with a variable mitotic rate and frequently express desmin; however, they lack a cambium layer or striated cells and do not express myogenin.

Alveolar Rhabdomyosarcoma

Alveolar RMS is histologic pattern composed of malignant small rounded cells that are typically discohesive with a tendency to attach to and line up along thin fibrous septa. The tumor cells have some variation in size. Tumor cell nuclei are round and lymphocyte-like with coarse chromatin and one or more indistinct nucleoli. Tumor cells may show a thin rim of eosinophilic cytoplasm. Morphologic evidence of rhabdomyoblastic differentiation including strap cells or cells with cross-striations is often lacking, although multinucleate myoblasts may be seen. It is important to recognize the "solid variant," in which the tumor cells grow in solid masses of closely aggregated cells. Classification as alveolar RMS is based on histologic features, as approximately 15 to 20% of all alveolar RMS will lack *FOXO1* fusion genes.

The differential diagnosis of alveolar RMS includes the panoply of malignant small round cell neoplasms, particularly Ewing sarcoma, poorly differentiated or undifferentiated neuroblastoma, desmoplastic small round cell tumor, poorly differentiated monophasic synovial sarcoma, and lymphoma. A panel of immunohistochemical stains including myogenin, desmin, MYOD1, cytokeratin, CD99, WT1, synaptophysin, chromogranin, and leukocyte common antigen (CD45) will distinguish alveolar RMS from these other entities, but unexpected staining with antigens such as cytokeratin may occur. In contrast to dense ERMS, ARMS shows strong diffuse staining with myogenin (typically >80%) and AP2beta, with weak to absent HMGA2. Molecular studies show *PAX3*- and *PAX7-FOXO1* fusion gene products occur in approximately 80-85% of alveolar RMS cases. Molecular testing is required for risk stratification in all alveolar RMS cases.

Spindle Cell/Sclerosing Rhabdomyosarcoma

In the 5th edition of *WHO Classification of Tumours of Soft Tissue and Bone*, spindle cell and sclerosing RMS are considered in the same diagnostic category.[4] Spindle cell / sclerosing RMS is uncommon, accounting for 3% to 10% of all cases of RMS. Spindle cell/sclerosing rhabdomyosarcoma includes three distinct genetic subtypes. First, in infants, spindle cell RMS is often associated with recurrent non-*FOXO1* gene fusions involving *VGLL2* or *NCOA2*; these are of unclear prognosis.[12,13] Initial studies demonstrated these tumors to have a favorable prognosis. However, a recent study showed a subset of tumors with a more aggressive biology including recurrence, metastasis and death from disease, to include late events.[13] This remains an evolving area with, to date, an uncertain overall prognosis. Second, *MYOD1* mutated spindle cell/sclerosing RMS occurs more frequently in adolescents and adults.[5] These tumors are more common in the head and neck region (particularly parameningeal) and are associated with a poor prognosis, including a recurrence and metastasis rate of 40%-50%.[14] One study of patients with *MYOD1* mutated RMS showed 68% died of disease.[15] Third, recent series describe an intraosseous

spindle cell RMS involving fusions of the *TFCP2* gene to either *EWSR1* or *FUS* genes, which also demonstrate immunoreactivity to keratins and ALK.[16,17] These tumors are also associated with a poor outcome, although there are few cases published to date.[16,17]

Of note, in children, a subset of spindle cell RMS located in the paratesticular region, do not have known recurrent genetic aberrations. Spindle cell RMS account for 26.7% of RMS in the paratesticular site, the remainder mostly being typical embryonal RMS; these spindle cell RMS may also represent a spindled variant of embryonal RMS.[18,19] The 5-year survival for patients with spindle cell RMS in the paratesticular location is excellent.[18,19]

Histologically, spindle cell/sclerosing RMS is somewhat variable. The spindle cell morphologic pattern is that of ovoid to fusiform spindle cells, arranged in fascicles or bundles, sometimes with a herringbone like growth pattern. Spindle cell RMS of infancy can have a more myoid appearance which can resemble a smooth muscle tumor. Some cases may contain rhabdomyoblastic differentiation; however, this tends to not be as pronounced as typically observed in embyronal RMS. Infantile spindle cell RMS and the spindled pattern of ERMS are exclusively spindled, without regions of sclerosis. In contrast, spindle or sclerosing patterns may be seen in *MYOD1* mutated tumors. Sclerosing RMS is characterized by a dense hyalinizing collagenous matrix with rounded or spindle-shaped tumor cells arranged in small nests, single-file rows, and pseudovascular, microalveolar profiles.[12,14,20] Spindle cell/sclerosing RMS may have only focal positivity for desmin and myogenin (myf4) but typically strongly expresses MYOD1 (myf3).

The primary differential diagnosis of spindle cell RMS includes embryonal RMS NOS, leiomyosarcoma, fibrosarcoma, undifferentiated spindle cell sarcoma, and the more bland entities, rhabdomyoma, leiomyoma, and nodular fasciitis. In general, smooth muscle neoplasms are uncommon in childhood and adolescence. The presence of specific skeletal muscle antigens (e.g., myoglobin, MYOD1, myogenin) and the ultrastructural presence of skeletal myofilaments or sarcomeric structures help in distinguishing spindle cell RMS from leiomyosarcoma, fibrosarcoma, and undifferentiated spindle cell sarcoma. The histologic differential for the sclerosing pattern RMS includes sclerosing epithelioid fibrosarcoma, infiltrating carcinoma, osteosarcoma, and angiosarcoma.

Ectomesenchymoma

Ectomesenchymoma is a rare malignant tumor that generally consists of an RMS component (embryonal greater than alveolar) and a ganglionic and/or neuroblastic component. The name originates from the belief that these tumors arise from pluripotent migrating neural crest cells or “ectomesenchyme.” They have a similar age, sex, and site distribution and outcome to embryonal RMS and are treated with RMS-based therapy. Ectomesenchymomas may be further subclassified based on the subtype of RMS seen.

Other

In very rare occasions, an alveolar RMS pattern can be seen in a tumor that would otherwise be classified as embryonal RMS. These mixed alveolar and embryonal tumors resemble “collision” tumors, with differential myogenin expression between alveolar and embryonal components.[5] These tumors may be fusion positive (most frequently *PAX7-FOXO1*) or fusion negative, although when tested separately each component shows the same genetic profile.

Posttreatment RMS may show extensive cytodifferentiation mimicking a highly differentiated embryonal RMS (see Note G).

RMS, Not Otherwise Specified

RMS, NOS, is reserved for cases in which a diagnosis of RMS can be made based on immunohistochemistry, but the case cannot be confidently further classified due to extensive necrosis, crush, or other aspect of the specimen that limits histologic interpretation.

Immunohistochemistry

In cases where histological diagnosis of rhabdomyosarcoma is difficult, immunostaining with monoclonal antibodies against the intranuclear myogenic transcription factors MYOD1 and myogenin, and the cytoplasmic intermediate filament desmin is suggested. Nearly all RMS tumors are positive for desmin, myogenin (nuclear), and MyoD1 (nuclear).[21,22] On occasion, anti-myogenin reacts with other spindle cell neoplasms,[23] and rare RMS cases may be myogenin negative and desmin positive.[24] Of note, desmin expression is frequent in certain round cell tumors, such as blastemal Wilms tumor, tenosynovial giant cell tumor, and desmoplastic small round cell tumor. Myogenin is more specific but may occur in rare lesions such as melanotic neuroectodermal tumor of infancy, as well as any lesion capable of skeletal myogenesis such as Wilms tumor, teratoma, pleuropulmonary blastoma, or malignant Triton tumor (malignant peripheral nerve sheath tumor with rhabdomyoblastic differentiation). Caution should also be taken when interpreting myogenin reactivity in tumors that interface with normal skeletal muscle, as injured muscle fibers can express myogenin.

References

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E. Anaplasia

Anaplasia is found in up to 16% of RMS and may be found in any histologic subtype.[1,2,3] Anaplastic tumors are defined as having large, lobate, hyperchromatic nuclei (at least 3 times the size of neighboring nuclei) or atypical (obvious, multipolar) mitotic figures.

Anaplasia is further described based on distribution of the anaplastic cells: focal (group I) anaplasia, which consists of a single or a few cells, scattered amongst nonanaplastic cells; or diffuse (group II), in which clusters or sheets of anaplastic cells are evident. These features should be visible at low power (10X objective) to avoid confusing it with “nuclear unrest,” characterized by mild degrees of hyperchromatism and nuclear atypia that do not qualify as 3X enlargement, do not contain atypical mitoses.[4] Care must also be taken to distinguish anaplasia from the changes of myogenic differentiation, ie, multinucleation, overlapping nuclei, and nuclear atypia. However, this can be avoided by identifying atypical, multipolar mitoses and using caution in cells with abundant cytoplasm.[5] Anaplasia is more common in tumors arising at favorable sites, and in stage 1 and clinical group I and II tumors.[2] A recent large study showed no difference in failure-free or overall survival in patients with RMS having no anaplasia, focal anaplasia or diffuse anaplasia, and anaplasia was not an independent adverse prognostic factor.[3] Anaplasia is associated with TP53 mutations, and 69% of tumors with TP53 mutations showed histologic anaplasia in this same series.[3] Because of the correlation between anaplastic embryonal RMS and TP53 mutations (both tumor and germline), screening for germline TP53 mutations may be indicated in these patients.[6]

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F. Margins

The extent of resection (i.e., gross residual disease versus complete resection) has the strongest influence on local control of malignancy.[1,2] The definition of what constitutes a sufficiently "wide" margin of normal tissue in the management of RMS has evolved over time from resection of the whole muscle to resection with a 2-3 cm margin.

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G. Relevant History

Relevant historical factors include any previous therapy, family history of malignancy, and the presence of congenital anomalies. If preoperative therapy has been given, assessment may be limited to the estimate of viable and necrotic RMS.[1] The tumor may also show extreme cytodifferentiation and nuclear pleomorphism. These factors may preclude accurate subtyping of the RMS.

There is a specific concern for increased risk of a familial cancer when the specific diagnosis of embryonal RMS or other soft tissue sarcoma is made within the first 2 years of life, especially in a male child.[2] Such syndromes include Li-Fraumeni syndrome, basal cell nevus syndrome, neurofibromatosis, and pleuropulmonary blastoma syndrome (pleuropulmonary blastoma plus malignancies associated with germline *DICER1* mutations).[1,3] Agenetic predisposition to cancer is thought to be present in 7%-33% of children with soft tissue sarcomas.[4,5]

Rhabdomyosarcoma is specifically associated with a variety of congenital anomalies.[6] These include congenital anomalies of the central nervous system, genitourinary tract, gastrointestinal tract, and cardiovascular system.

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H. Fusion Status

The presence of a t(1;13) (resulting in a *PAX7-FOXO1* (*FKHR*) gene fusion) or a t(2;13) (*PAX3-FOXO1* gene fusion) is strongly correlated with the alveolar subtype of rhabdomyosarcoma. These translocations may be found in as many as 85% of alveolar RMS cases, while embryonal RMS cases lack evidence of these gene fusions (with rare exceptions).[1] Some tumors with alveolar histology lack a demonstrable *PAX* fusion. By gene expression profiling, they do not cluster with *PAX* fusion-positive tumors and have a genetic signature that more closely resembles embryonal RMS.[2,3] Recent studies have confirmed that the presence of a *PAX-FOXO1* fusion transcript drives outcome in children with rhabdomyosarcoma.[4,5] Accordingly, future cooperative group studies conducted by both the Children's Oncology Group and European Pediatric Soft Tissue Sarcoma Group will use *FOXO1* fusion status rather than alveolar histology to assign risk stratification and treatment for patients with RMS. Fusion status is therefore a required element for all patients with alveolar rhabdomyosarcoma. In contrast, embryonal and non-alveolar patterns of rhabdomyosarcoma are nearly always *FOXO1* fusion negative and testing is not required. However, fusion studies can be extremely useful in cases with limited or questionable material, those in which histologic classification is difficult or those with unusual clinical characteristics (e.g., embryonal subtype arising in an extremity).[6] *PAX-FOXO1* gene fusions have also been described in mixed alveolar and embryonal rhabdomyosarcoma and ectomesenchymoma with an alveolar RMS component.

Of fusion-positive RMS cases, approximately 30% are positive for *PAX7-FOXO1*, and the remaining 70% are positive for *PAX3-FOXO1*. If RT-PCR using *PAX3*- or *PAX7*-specific probes is not used to determine fusion status, amplification of *FOXO1* on break-apart FISH studies can act as a surrogate marker of *PAX7-FOXO1* fusion status.[7] Studies suggest that patients with alveolar RMS expressing the *PAX3-FOXO1* gene product have a lower event-free survival than *PAX7-FOXO1*-positive alveolar RMS,[8] but the significance of the translocations must still be elucidated. Some data indicate that when gene fusion status is compared in patients with metastatic disease at diagnosis, a striking difference in outcome is seen between *PAX7-FOXO1* and *PAX3-FOXO1* (estimated 4-year overall survival of 75% for *PAX7-FOXO1* and 8% for *PAX3-FOXO1*; P=.002).[9]

Although rare, several variant fusion transcripts have been described in alveolar RMS. Most include fusion of *PAX3* with an alternate partner, such as *NCOA1*, *NCOA2*, or *FOXO4*. Less often *FOXO1* is preserved and fused with another partner, such as *FGFR1*. Due to the low incidence of these variant fusion transcripts, the prognostic significance is unknown. Some evidence suggests different fusion transcripts may confer different prognostic effects,[10] but until more is known these tumors are treated under fusion-positive RMS protocols.

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I. Molecular Genetic Studies

As noted in the histologic types section (Note D), some molecular alterations may be associated with particular RMS subtypes. Additionally, some alterations may have prognostic or germline implications. *DICER1* mutations are associated with a subset of embryonal RMS and may be somatic or germline; identification of a *DICER1* mutation in an embryonal RMS tumor warrants additional exploration for the possibility of *DICER1* syndrome/PPB-tumor predisposition syndrome (germline mutations associated with PPB, ERMS, cystic nephroma, sex cord stromal tumors, pineal blastoma, among other tumors).[1] In spindle cell / sclerosing RMS, *MYOD1 L122R* point mutations are of prognostic significance, with those tumors containing these alterations demonstrating a more aggressive biology.[2] Both *DICER1* and *MYOD1* alterations are point mutations and the sensitivity for detecting these mutations in next-generation sequencing panels designed to detect RNA fusions may be variable; detection of these mutations using targeted DNA sequencing may be considered as an alternative. In spindle cell RMS in infants, detection of a *VGLL2* or *NCOA2* gene fusion may be helpful in diagnosis as well as predictive of a favorable prognosis. Intraosseous spindle cell RMS is an evolving diagnostic area; identification of either a *EWSR1-TFCP2* or *FUS-TFCP2* fusion may aid in the diagnosis of this rare subtype.[3,4,5]

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