Protocol for the Examination of Biopsy Specimens From Pediatric Patients With Rhabdomyosarcoma

Version: Rhabdomyosarcoma Biopsy 4.0.0.0  Protocol Posting Date: February 2019
Includes the Intergroup Rhabdomyosarcoma Study Postsurgical Clinical Grouping System

Accreditation Requirements
The use of this protocol is recommended for clinical care purposes but is not required for accreditation purposes.

This protocol should be used for the following procedures AND tumor types:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>Includes specimens designated core biopsy, incisional biopsy, excisional biopsy, or other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhabdomyosarcoma</td>
<td>Includes pediatric patients with all rhabdomyosarcoma variants and ectomesenchymoma</td>
</tr>
</tbody>
</table>

The following should NOT be reported using this protocol:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resection</td>
<td>(consider Rhabdomyosarcoma Resection protocol)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Rhabdomyosarcoma*</td>
<td>(consider using soft tissue protocol)</td>
</tr>
</tbody>
</table>

*Rhabdomyosarcoma in adults may be treated differently than pediatric rhabdomyosarcoma, and use of the AJCC TNM staging system remains appropriate for these patients.

Authors
Erin R. Rudzinski, MD*; Armita Bahrami, MD; David M. Parham, MD; Neil Sebire
With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees

* Denotes primary author. All other contributing authors are listed alphabetically.

Important Note
First priority should always be given to formalin-fixed tissue for morphologic evaluation. Optimally, at least 100 mg of viable snap-frozen tissue is preferred as the second priority for workup (Note A).

For more information, contact: The Children’s Oncology Group Biopathology Center; Phone: (614) 722-2890 or (800) 347-2486.

Summary of Changes
v4.0.0.0 - Biopsy and resection procedures separated into individual protocols
### Surgical Pathology Cancer Case Summary

Protocol posting date: February 2019

**RHABDOMYOSARCOMA AND RELATED NEOPLASMS: Biopsy**

**Note:** This case summary is recommended for reporting Rhabdomyosarcoma but is NOT REQUIRED for accreditation purposes. Core data elements are bolded to help identify routinely reported elements.

Select a single response unless otherwise indicated.

**Procedure (Note B)**
- ___ Core needle biopsy
- ___ Incisional biopsy
- ___ Incisional biopsy
- ___ Other (specify): ________________________
- ___ Not specified

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

**Tumor Size (for excisional biopsy only)**
- Greatest dimension: (centimeters) ___ cm
- Additional dimensions: (centimeters) ___ x ___ cm
- ___ Cannot be determined (explain): ______________________________

**Histologic Type (Note C)**
- ___ Embryonal
- ___ Alveolar
- ___ Spindle cell/sclerosing
- ___ Ectomesenchymoma
- ___ Rhabdomyosarcoma, not otherwise specified (NOS)
- ___ Other (specify): ____________________________

**Anaplasia (Note D)**
- ___ Not identified
- ___ Focal (single or few scattered anaplastic cells)
- ___ Diffuse (clusters or sheets of anaplastic cells)
- ___ Cannot be determined

**Margins (for excisional biopsy only) (Note E)**
- ___ Cannot be assessed
- ___ Uninvolved by tumor
  - Distance of tumor from closest margin (centimeters): ___ cm
  - Specify margin: ______________________________
- ___ Involved by tumor
  - Specify margin(s): ____________________________

The routinely reported core data elements are bolded.
Fusion Status (Note F)
___ Not performed
___ Pending
___ No FOXO1 rearrangement
___ FOXO1 rearrangement present (if known, select all that apply)
   ___ Amplification status (ie, fluorescence in situ hybridization [FISH]) (specify): __________________
       ___ PAX3
       ___ PAX7
___ Other (eg, PAX3-NCOA1 or other variant translocation) (specify): __________________________

Method
___ Karyotype
___ FISH
___ Reverse transcriptase polymerase chain reaction (RT-PCR)
___ Other (specify): __________________________

Additional Pathologic Findings (Note G)
Specify: ______________________________

Comment(s)
Explanatory Notes

A. Submission of Tissue
A minimum of 100 mg of viable tumor should be snap-frozen for potential molecular studies. 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:

B. 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. Open incisional biopsy consistently provides a larger sample of tissue and maximizes the opportunity for a specific pathologic diagnosis. Excisional biopsy may not include an adequate margin of normal tissue, even with an operative impression of total gross removal.

References:

C. Histologic Type
The International Classification of Rhabdomyosarcoma classified childhood rhabdomyosarcoma (RMS) into prognostically useful histologic categories. However, recent studies showed that fusion status drives unfavorable outcome for children with rhabdomyosarcoma, and histologic classification is no longer the primary tool for determining prognosis and risk stratification. The 4th edition of WHO Classification of Tumours of Soft Tissue and Bone limits the histologic classification of rhabdomyosarcoma to 4 categories: embryonal (including botryoid), alveolar, spindle cell/sclerosing, and pleomorphic subtypes. 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 characterized an epithelioid/rhabdoid pattern of RMS. 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 histologic classification.

Embryonal Rhabdomyosarcoma
Embryonal RMS includes the typical (or not otherwise specified), 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; its myogenin immunostaining pattern (focal, not diffuse) and testing for PAX-FOXO1 translocations may assist in making this distinction. Perivascular condensations of tumor cells in the less cellular regions are common.

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. 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.

Epithelioid (or rhabdoid-like) RMS is a rare type of RMS that shows abundant cells with large amounts of eosinophilic cytoplasm and intermediate-filament globular inclusions similar to those seen in malignant rhabdoid tumors (MRTs). Tumors differ from MRT in their nuclear cytologic features; in rhabdoid RMS, the nuclear chromatin tended to be coarse instead of vesicular. Immunohistochemically, the inclusions were positive for vimentin and desmin, and the cytoplasm adjacent to the inclusion was positive for muscle specific actin and desmin. The outcome in this group seems similar to other non-alveolar subtypes of RMS. Pure epithelioid RMS may resemble poorly differentiated squamous carcinoma or epithelioid sarcoma. Myogenin and INI-1 staining may be helpful in making the distinction between this neoplasm and true rhabdoid tumor or epithelioid sarcoma. Epithelioid RMS will show nuclear myogenin expression (negative in MRT) and retained expression of INI-1 (lost in MRT).

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, however. Ectomesenchymoma (discussed below) typically has embryonal RMS along with a neuroblastic/ganglion cell component. Undifferentiated embryonal sarcoma of the liver has some morphologic and phenotypic overlap, but it generally does not express MyoD1 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 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. Large, multinucleate cells can be found occasionally. 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. Of note, many if not most “solid variant” alveolar RMS lack evidence of a PAX fusion and are biologically more akin to embryonal RMS. With wide sampling, areas showing cleft-like spaces or a more classically alveolar pattern can usually be found, facilitating recognition of these tumors as alveolar RMS.

The differential diagnosis of alveolar RMS includes the panoply of malignant small round cell neoplasms, particularly Ewing sarcoma/primitive neuroectodermal tumor, 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, Myo-D1, cytokeratin, CD99, WT1, synaptophysin, chromogranin, and leukocyte common antigen will distinguish alveolar RMS from these other entities, but unexpected staining with antigens such as cytokeratin may occur. Alveolar RMS shows diffuse and strong nuclear staining for myogenin. Molecular studies show PAX3- and PAX7-FOXO1 fusion gene products
occur in approximately 85% of alveolar RMS cases. Molecular testing is required for risk stratification in all alveolar RMS cases.

**Spindle Cell/Sclerosing Rhabdomyosarcoma**

In the 4th edition of *WHO Classification of Tumours of Soft Tissue and Bone*, spindle cell/sclerosing RMS are considered in the same diagnostic category based on their predilection for the head and neck/extremities and similar clinical behavior. Both spindle cell and sclerosing RMS are uncommon, together accounting for 5% to 10% of all cases of RMS. Recent studies suggest that spindle cell/sclerosing rhabdomyosarcoma includes three distinct biologic subtypes. In infants, spindle cell RMS is often associated with recurrent non-PAX gene fusions involving VGLL2 or NCOA2, and these tumors are associated with a good prognosis. In children, almost one-third of spindle cell RMS are located in the paratesticular region, where they account for 26.7% of RMS in this site, the remainder mostly being typical embryonal RMS. The 5-year survival for patients with spindle cell RMS in the paratesticular location is excellent, at 88%. However, the favorable prognosis of spindle cell RMS does not apply to lesions outside the paratesticular region, as tumors in these other locations have a prognosis similar to typical embryonal RMS in children. In adolescents and adults spindle cell/sclerosing RMS has a recurrence and metastasis rate of 40%-50%. These tumors are often parameningeal in location and are associated with recurrent MYOD1 mutations. One study of patients with MYOD1 mutated RMS showed 68% died of disease.

Spindle cell RMS is composed almost exclusively (minimum 80% of tumor) of elongated spindle cells in 1 of 2 recognizable patterns. The collagen-poor pattern has a whorled, fascicular growth of spindle cells without significant collagen and resembles a smooth muscle tumor both grossly and microscopically. The collagen-rich form shows spindle cells with variable myogenic differentiation in a dense collagenous stroma. The spindle cells have eosinophilic, fibrillar cytoplasm with distinct borders. Cells with cross-striations are easily found. A small component (less than 20%) of typical embryonal RMS may be seen in some cases, usually at the tumor periphery. Anaplasia is uncommon.

The primary differential diagnosis of spindle cell RMS includes embryonal RMS NOS, leiomyosarcoma, fibrosarcoma, malignant fibrous histiocytoma (MFH), 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 (eg, myoglobin, MyoD1, myogenin) and the ultrastructural presence of skeletal myofilaments help in distinguishing spindle cell RMS from leiomyosarcoma, fibrosarcoma, and MFH.

Sclerosing RMS is most common in the extremities, where differentiation from alveolar RMS is important. 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. As with spindle cell RMS, this should be the predominant pattern, present in at least 80% of the tumor. Sclerosing RMS may have only focal positivity for desmin and myogenin (myf4) but typically strongly expresses MyoD1 (myf3). This pattern has morphologic overlap with sclerosing epithelioid fibrosarcoma, infiltrating carcinoma, osteosarcoma, and angiosarcoma. Spindle cell/sclerosing RMS should be PAX-fusion negative and has constituted some “fusion-negative alveolar RMS” in previous studies. Cytogenetic studies have described aneuploidy and nonrecurrent structural changes. Recent studies have demonstrated recurrent MyoD1 mutations in spindle cell RMS.

**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. These tumors may be fusion positive or fusion negative, although when tested separately each component shows the same genetic profile.
Posttreatment RMS may show extensive cytodifferentiation mimicking epithelioid/rhabdoid RMS or 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 further classified due to extensive necrosis, crush, or other artifact.

Immunohistochemistry
In cases where histological diagnosis of rhabdomyosarcoma is difficult, immunostaining with monoclonal antibodies against the intranuclear myogenic transcription factors MyoD1, myogenin, and desmin is suggested. Nearly all RMS tumors are positive for desmin, myogenin, and MyoD1.15,16 On occasion, anti-myogenin reacts with other spindle cell neoplasms,17 and rare RMS cases may be myogenin negative and desmin positive.18 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, and it occurs infrequently in primitive neuroectodermal 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 nephroblastoma (Wilms tumor), teratoma, pleuropulmonary blastoma, or malignant Triton tumor (malignant peripheral nerve sheath tumor with rhabdomyoblastic differentiation).

Immunohistochemistry may be useful as a surrogate marker for fusion status in rhabdomyosarcoma and aids in the diagnosis of alveolar RMS. Several studies show that AP2beta is highly sensitive and specific for the detection of fusion-positive RMS.18-20 Immunohistochemistry for other antibodies (NOS-1 and HMGA2) in addition to AP2beta may improve the sensitivity for detection of fusion-positive RMS and may aid in the detection of tumors with rare fusion variant translocations (discussed below).21

References:
D. Anaplasia

Anaplasia is found in up to 13% of RMS and may be found in any histologic subtype. Anaplastic tumors are defined using the Wilms tumor definition of large, lobate, hyperchromatic nuclei (at least 3 times the size of neighboring nuclei) and atypical (obvious, multipolar) mitotic figures.

Anaplasia is further defined as to the distribution of the 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 bizarre mitoses, and do not affect outcome to the same degree. 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. Anaplasia is more common in patients with tumors in favorable sites and less commonly observed in younger patients and in those with stage II, III, or clinical group III disease.

Regardless of focal or diffuse distribution, the presence of anaplasia negatively influences the failure-free survival rate (63% versus 77% at 5 years) and overall survival (68% versus 82% at 5 years) rates in patients with embryonal rhabdomyosarcoma. This effect is most pronounced in children with intermediate-risk tumors but does not affect outcome in patients with alveolar tumors. Although it has predictive value for clinical outcome, current treatment protocols do not account for anaplasia in stratification of patients, as it has limited value as an independent survival marker when all other prognostic factors are considered. Because of the correlation between anaplastic embryonal RMS and Li-Fraumeni syndrome, screening for germline TP53 mutations may be indicated in these patients.

References:


**E. Margins**

The extent of resection (ie, gross residual disease versus complete resection) has the strongest influence on local control of malignancy. 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.

References:


**F. Fusion Status**

The presence of a t(1;13) (resulting in a PAX7-FOXO1 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). Some tumors with alveolar histology lack a demonstrable PAX fusion. By gene array testing, they do not cluster with PAX fusion-positive tumors and have a genetic signature that more closely resembles embryonal RMS. Recent studies confirmed that presence of a PAX-FOXO1 fusion transcript drives outcome in children with rhabdomyosarcoma. Accordingly, future cooperative group studies conducted by both the Children’s Oncology Group and European Pediatric Soft Tissue Sarcoma Group will use 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 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 (eg, embryonal subtype arising in an extremity). 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. Studies suggest that patients with alveolar RMS expressing the PAX3-FKHR gene product have a lower event-free survival than PAX7-FKHR-positive alveolar RMS, 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-FKHR and PAX3-FKHR (estimated 4-year overall survival of 75% for PAX7-FKHR and 8% for PAX3-FKHR; P=.002).

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, but until more is known these tumors are treated under fusion-positive RMS protocols.

References:


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. 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. Such syndromes include Li-Fraumeni syndrome, basal cell nevus syndrome, neurofibromatosis, and pleuropulmonary blastoma syndrome (pleuropulmonary blastoma plus associated malignancies). A genetic predisposition to cancer is thought to be present in 7%-33% of children with soft tissue sarcomas.

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

References: