Protocol for the Examination of Resection Specimens From Patients With Primary Tumors of Bone

Version: 4.2.0.0
Protocol Posting Date: June 2024
CAP Laboratory Accreditation Program Protocol Required Use Date: March 2025

The changes included in this current protocol version affect accreditation requirements. The new deadline for implementing this protocol version is reflected in the above accreditation date.

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>Resection</td>
<td>Includes specimens designated intralesional resection, marginal resection,</td>
</tr>
<tr>
<td></td>
<td>segmental resection / limb salvage, wide resection, or radical resection /</td>
</tr>
<tr>
<td></td>
<td>amputation.</td>
</tr>
<tr>
<td>Tumor Type</td>
<td>Description</td>
</tr>
<tr>
<td>Primary malignant bone tumors</td>
<td>Includes tumors arising in bone for which pTNM staging is clinically relevant.</td>
</tr>
</tbody>
</table>

This protocol is NOT required for accreditation purposes for the following:

<table>
<thead>
<tr>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy (includes core, curettage, or incisional, consider Bone Biopsy Protocol)</td>
</tr>
<tr>
<td>Primary resection specimen with no residual / viable cancer (e.g., following neoadjuvant therapy)</td>
</tr>
<tr>
<td>Cytologic fine needle aspiration (FNA) specimens</td>
</tr>
<tr>
<td>Tumor Type</td>
</tr>
<tr>
<td>Bone tumors that may recur locally but have either no or an extremely low risk of metastasis</td>
</tr>
</tbody>
</table>

The following tumor types should NOT be reported using this protocol:

<table>
<thead>
<tr>
<th>Tumor Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoma / Leukemia (consider the Precursor and Mature Lymphoid Malignancies, Myeloid and Mixed / Ambiguous Lineage Neoplasms, or Plasma Cell Malignancies protocols)</td>
</tr>
<tr>
<td>Pediatric Ewing sarcoma (consider the Pediatric Ewing Sarcoma protocol)</td>
</tr>
<tr>
<td>Soft tissue primary sarcoma (consider the Soft Tissue protocol)</td>
</tr>
</tbody>
</table>

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With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.
* Denotes primary author.
Accreditation Requirements
This protocol can be utilized for a variety of procedures and tumor types for clinical care purposes. For accreditation purposes, only the definitive primary cancer resection specimen is required to have the core and conditional data elements reported in a synoptic format.

- **Core data elements** are required in reports to adequately describe appropriate malignancies. For accreditation purposes, essential data elements must be reported in all instances, even if the response is “not applicable” or “cannot be determined.”
- **Conditional data elements** are only required to be reported if applicable as delineated in the protocol. For instance, the total number of lymph nodes examined must be reported, but only if nodes are present in the specimen.
- **Optional data elements** are identified with “+” and although not required for CAP accreditation purposes, may be considered for reporting as determined by local practice standards.

The use of this protocol is not required for recurrent tumors or for metastatic tumors that are resected at a different time than the primary tumor. Use of this protocol is also not required for pathology reviews performed at a second institution (i.e., secondary consultation, second opinion, or review of outside case at second institution).

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 4.2.0.0

- Cover page update
- Updates to content and explanatory notes, including WHO Histologic Types
- pTNM Classification update
- LVI question update from optional to required (core) and “Lymphovascular Invasion” to “Lymphatic and / or Vascular Invasion”
- “Other Close Margin(s) to Tumor” criterion update
- “Mitotic Rate” answer update
- Addition of required (core) questions “Treatment Effect (for post-neoadjuvant therapy)”, and “Tumor Laterality”
- Updates to conditional question “Necrosis in the Absence of Neoadjuvant therapy (required only if neoadjuvant therapy was not administered)”
- SPECIAL STUDIES section update
CASE SUMMARY: (BONE: Resection)
Standard(s): AJCC-UICC 8

CLINICAL (Note A)

+Associated Syndrome
___ Li-Fraumeni syndrome
___ Mazabraud syndrome
___ Ollier disease
___ Maffucci syndrome
___ Hereditary multiple exostoses
___ Other (specify): _________________
___ Not specified

+Radiologic Findings (Notes A,B)
___ Specify: _________________
___ Not available

+Preresection Treatment (select all that apply)
___ No known preresection therapy
___ Chemotherapy
___ Radiation therapy
___ Other (specify): _________________
___ Therapy administered, type not specified
___ Not specified

+Other Clinical Findings
___ Specify: _________________
___ Not available

SPECIMEN

Procedure (Note C)
___ Intralesional resection
___ Marginal resection
___ Segmental / wide resection
___ Limb salvage (specify): _________________
___ Radical resection of bone and soft tissue
___ Amputation (specify): _________________
___ Other (specify): _________________
___ Not specified
+Decalcification Procedure (Note C)
___ EDTA-decal or equivalent
___ Harsh acid decalcification

TUMOR

Multiple Sites (required only if applicable)
___ Not applicable
___ Multifocal tumor / discontinuous tumor at primary bone site
___ Additional primary bone site(s) present (specify for synchronous malignant tumors or polyostotic aggressive tumors): __________________

Tumor Site (Note D)
___ Appendicular skeleton, trunk, skull, facial bones (specify): __________________
___ Spine (specify bone, if known): __________________
___ Pelvis (specify bone, if known): __________________
___ Not specified

Tumor Laterality
___ Left
___ Right
___ Central
___ Polyostotic ipsilateral
___ Polyostotic bilateral
___ Cannot be determined

Tumor Location and Extent (Note B) (select all that apply)
___ Epiphysis or apophysis
___ Metaphysis
___ Diaphysis
___ Cortex
___ Medullary cavity
___ Surface
___ Involves joint
___ Extends into soft tissue
___ Cannot be determined: __________________

Tumor Size
___ Greatest dimension in Centimeters (cm): ____________ cm
___ Additional Dimension in Centimeters (cm): ____ x ____ cm
___ Radiologic Greatest Dimension in Centimeters (cm): ____________ cm
___ Cannot be determined: __________________
## Histologic Type# (Note E)

The list is derived from the World Health Organization (WHO) classification of bone tumors, 5th edition, to include ONLY bone tumors of intermediate (locally aggressive and rarely metastasizing) potential and malignant bone tumors.

### Chondrogenic tumors
- Synovial chondromatosis
- Atypical cartilaginous tumor
- Chondrosarcoma
- Chondrosarcoma, secondary (specify): _________________
- Dedifferentiated chondrosarcoma
- Periosteal chondrosarcoma
- Clear cell chondrosarcoma
- Mesenchymal chondrosarcoma

### Osteogenic tumors
- Osteoblastoma
- Low-grade central osteosarcoma
- Low-grade central osteosarcoma with high-grade transformation
- Parosteal osteosarcoma
- Parosteal osteosarcoma with high-grade transformation
- Conventional osteosarcoma
- Telangiectatic osteosarcoma
- Small cell osteosarcoma
- Periosteal osteosarcoma
- High-grade surface osteosarcoma
- Secondary osteosarcoma

### Precipitating Factor for Secondary Osteosarcoma: _________________

### Undifferentiated small round cell sarcomas
- Ewing sarcoma
- Round cell sarcoma with EWSR1::non-ETS fusions (specify, if known): _________________
- CIC-rearranged sarcoma
- Sarcoma with BCOR genetic alterations

### Fibrogenic / fibrohistiocytic / histiocytic tumors
- Sclerosing epithelioid fibrosarcoma
- Primary malignant giant cell tumor of bone
- Secondary malignant giant cell tumor of bone
- Giant cell tumor of bone
- Langerhans cell histiocytosis

### System Involvement
- Single system (specify): _________________
- Multisystem (specify): _________________
- Other (leukemic, atypical, or other, specify): _________________
- Desmoplastic fibroma

### Notochordal tumors
- Conventional chordoma
- Poorly differentiated chordoma
- Dedifferentiated chordoma
- Vascular tumors
___ Epithelioid hemangioma
___ Pseudomyogenic hemangioendothelioma
___ Epithelioid hemangioendothelioma
___ Angiosarcoma
___ Epithelial tumors
___ Adamantinoma of long bones
___ Osteofibrous dysplasia-like adamantinoma
___ Dedifferentiated adamantinoma
___ Other mesenchymal tumors or tumors of uncertain differentiation
___ Leiomyosarcoma of bone
___ Rhabdomyosarcoma of bone (specify fusion, if known): _________________
___ TK-fusion (NTRK, ALK, BRAF) tumor, primary intraosseous (specify fusion, if known):
___ Undifferentiated pleomorphic sarcoma
___ Cannot be determined: _________________
___ Other histologic type not listed (specify): _________________
+Histologic Type Comment: _________________

Histologic Grade (Note F)
___ G1, well-differentiated, low-grade
___ G2, moderately differentiated, high-grade
___ G3, poorly differentiated, high-grade
___ GX, cannot be assessed: _________________
___ Ungraded tumor / not applicable for this tumor type

+Mitotic Rate (Note G)
___ Specify mitotic rate per mm2: _________________ mitoses per mm2
___ Specify mitotic rate per 10 high-power fields (HPF): _________________ mitoses per 10 high-power fields (HPF)
___ Cannot be determined (explain): _________________

Treatment Effect (for post-neoadjuvant treatment) (Note H)
___ No known presurgical therapy
___ Not identified
# Therapy response is expressed as a percentage of total tumor area that is non-viable.
___ Present (specify overall percentage of treatment effect)#: _________________ %
Select all that apply
+___ Geographic necrosis
+___ Fibrosis
+___ Hyalinization
+___ Hemorrhage
+___ Cystic change
+___ Histiocytic response
+___ Inflammation
+___ Other (specify): _________________
___ Cannot be determined
Necrosis in the Absence of Neoadjuvant Therapy (required only if neoadjuvant therapy was not administered)
___ Not applicable (neoadjuvant therapy was administered)
___ Not identified
___ Present

**Extent of Necrosis**
___ Specify percentage: _________________ %
___ Other (specify): _________________
___ Cannot be determined (explain): _________________
___ Cannot be determined

Lymphatic and / or Vascular Invasion (Note I)
___ Not identified
___ Present
___ Cannot be determined

+Tumor Comment: _________________

MARGINS (Note J)

Margin Status
___ All margins negative for tumor

**Closest Margin(s) to Tumor**
___ Specify closest margin(s): _________________
___ Cannot be determined (explain): _________________

**Distance from Tumor to Closest Margin**
Specify in Centimeters (cm)
___ Exact distance: _________________ cm
___ Greater than: _________________ cm
___ At least: _________________ cm
___ Less than: _________________ cm
___ Other (specify): _________________
___ Cannot be determined: _________________

+Other Close Margin(s) to Tumor (less than 0.2 cm)
___ Specify other close margin(s): _________________
___ Cannot be determined (explain): _________________
___ Not applicable
___ Tumor present at margin

**Margin(s) Involved by Tumor**
___ Specify involved margin(s): _________________
___ Cannot be determined: _________________
___ Other (specify): _________________
___ Cannot be determined (explain): _________________
___ Not applicable

+Margin Comment: _________________
REGIONAL LYMPH NODES (Note K)

Regional Lymph Node Status
___ Not applicable (no regional lymph nodes submitted or found)
___ Regional lymph nodes present
   ___ All regional lymph nodes negative for tumor
   ___ Tumor present in regional lymph node(s)

   Number of Lymph Nodes with Tumor
     ___ Exact number (specify): ___________________
     ___ At least (specify): ___________________
     ___ Other (specify): ___________________
     ___ Cannot be determined (explain): ___________________
     ___ Other (specify): ___________________
     ___ Cannot be determined (explain): ___________________

Number of Lymph Nodes Examined
___ Exact number (specify): ___________________
___ At least (specify): ___________________
___ Other (specify): ___________________
___ Cannot be determined (explain): ___________________

+Regional Lymph Node Comment: ___________________

DISTANT METASTASIS

Distant Site(s) Involved, if applicable (select all that apply)
___ Not applicable
___ Lung: ___________________
___ Bone: ___________________
___ Other (specify): ___________________
___ Cannot be determined: ___________________

pTNM CLASSIFICATION (AJCC 8th Edition) (Note L)
Reporting of pT, pN, and (when applicable) pM categories is based on information available to the pathologist at the time the report is issued. As per the AJCC (Chapter 1, 8th Ed.) it is the managing physician’s responsibility to establish the final pathologic stage based upon all pertinent information, including but potentially not limited to this pathology report.

pTNM Classification (required only if applicable)
# Regardless of the anatomic site, certain specific types of bone neoplasms for which pTNM staging is not clinically relevant are excluded from the staging system.
___ Not applicable (histologic type not appropriate for staging)#
___ Histologic type appropriate for staging

Modified Classification (required only if applicable) (select all that apply)
___ Not applicable
___ y (post-neoadjuvant therapy)
___ r (recurrence)
**pT Category**

___ Appendicular skeleton, trunk, skull, and facial bones

**pT Category**

___ pT not assigned (cannot be determined based on available pathological information)
___ pT0: No evidence of primary tumor
___ pT1: Tumor less than or equal to 8 cm in greatest dimension
___ pT2: Tumor greater than 8 cm in greatest dimension
___ pT3: Discontinuous tumors in the primary bone site

**Spine**

___ pT not assigned (cannot be determined based on available pathological information)
___ pT0: No evidence of primary tumor
___ pT1: Tumor confined to one vertebral segment or two adjacent vertebral segments
___ pT2: Tumor confined to three adjacent vertebral segments
___ pT3: Tumor confined to four or more adjacent vertebral segments, or any nonadjacent vertebral segments

**pT4**: Extension into the spinal canal or great vessels
___ pT4a: Extension into the spinal canal
___ pT4b: Evidence of gross vascular invasion or tumor thrombus in the great vessels
___ pT4 (subcategory cannot be determined)

**Pelvis**

___ pT not assigned (cannot be determined based on available pathological information)
___ pT0: No evidence of primary tumor
___ pT1: Tumor confined to one pelvic segment with no extraosseous extension

**pT1**: Tumor confined to one pelvic segment with no extraosseous extension
___ pT1a: Tumor less than or equal to 8 cm in greatest dimension
___ pT1b: Tumor greater than 8 cm in greatest dimension
___ pT1 (subcategory cannot be determined)

**pT2**: Tumor confined to one pelvic segment with extraosseous extension or two segments without extraosseous extension
___ pT2a: Tumor less than or equal to 8 cm in greatest dimension
___ pT2b: Tumor greater than 8 cm in greatest dimension
___ pT2 (subcategory cannot be determined)

**pT3**: Tumor spanning two pelvic segments with extraosseous extension
___ pT3a: Tumor less than or equal to 8 cm in greatest dimension
___ pT3b: Tumor greater than 8 cm in greatest dimension
___ pT3 (subcategory cannot be determined)

**pT4**: Tumor spanning three pelvic segments or crossing the sacroiliac joint
___ pT4a: Tumor involves sacroiliac joint and extends medial to the sacral neuroforamen
___ pT4b: Tumor encasement of external iliac vessels or presence of gross tumor thrombus in major pelvic vessels
___ pT4 (subcategory cannot be determined)

**T Suffix (required only if applicable)**

___ Not applicable
___ (m) multiple primary synchronous tumors in single anatomic site

**pN Category (Note K)**

___ pN not assigned (no nodes submitted or found)
___ pN not assigned (cannot be determined based on available pathological information)
___ pN0: No regional lymph node metastasis
___ pN1: Regional lymph node metastasis
  
**pM Category (required only if confirmed pathologically)**
___ Not applicable - pM cannot be determined from the submitted specimen(s)
    ___ pM1: Distant metastasis
      ___ pM1a: Lung
      ___ pM1b: Bone or other distant sites
      ___ pM1 (subcategory cannot be determined)

**ADDITIONAL FINDINGS**

+Additional Findings (specify): _________________

**SPECIAL STUDIES (Note E)**

*The previously reported biopsy immunohistochemistry, cytogenetics, and molecular studies can be included in the resection report.*

**Immunohistochemistry**
___ Specify results: _________________
___ Pending (specify): _________________
___ Not performed: _________________
___ Not applicable
___ Other (specify): _________________

**Cytogenetics**
___ Specify results: _________________
___ Pending (specify): _________________
___ Not performed: _________________
___ Not applicable
___ Other (specify): _________________

**Molecular Studies**
___ Specify results: _________________
___ Pending (specify): _________________
___ Not performed: _________________
___ Not applicable
___ Other (specify): _________________

**COMMENTS**

Comment(s): _________________
Explanatory Notes

A. Scope of Guidelines
Anatomical staging using the AJCC system 8th ed. is considered clinically relevant only for the specific malignant entities listed in World Health Organization classification 5th ed. This includes malignant chondrogenic tumors, osteogenic tumors, fibrogenic tumors, osteoclastic giant cell-rich tumors, notochordal tumors, myogenic tumors, lipogenic tumors, undifferentiated small round cell sarcomas and other mesenchymal tumors arising in bone. Locally aggressive entities such as synovial chondromatosis, osteoblastoma, giant cell tumor of bone, epithelioid hemangioma, pseudomyogenic hemangioma, and desmoplastic fibroma may be reported using this protocol but are not staged. Radiologic parameters include bone involved, size and extent (compartment) of tumor, location of tumor and extent, radiologic intrinsic characteristics including matrix or mineralization in bone-forming tumors, and differential diagnosis. Clinical parameters include patient age, sex, exact anatomic location, size, solitary or polyostotic, syndromes, and other pertinent medical and surgical history, if clinically relevant.

B. Tumor Location and Extent
Radiographic imaging plays an especially critical role in the diagnosis of bone tumors. Close collaboration with an experienced musculoskeletal radiologist and orthopedic surgeon is advised.

Figure 1 is a diagrammatic representation of the “anatomic” regions of a long bone. These locations are very important in classifying bone tumors. For instance, chondroblastoma almost always arises in the epiphysis. Epiphyses and apophyses are secondary ossification centers and therefore are embryonic equivalents; “epiphyses” are found within joints, whereas “apophyses”, the sites of tendonous and ligamentous attachments, are not found within joints. The greater and lesser trochanters are apophyses, while the epiphyses are at the ends of long bones.

Figure 1. Important anatomic landmarks for tumor diagnosis in long bones. Adapted from Gray’s Anatomy.1
C. Procedure/Tissue Processing/Tissue for Genetic-Molecular Studies

The following is a list of guidelines to be used in defining what type of procedure has been performed. This is based on the surgeon’s intent and not based on the pathologic assessment of the margins.

Intralesional Resection: Leaving gross tumor behind. Partial debulking is an example.

Marginal Resection: Removing the tumor and its pseudocapsule with a relatively small amount of adjacent tissue. There is no gross tumor at the margin; however, microscopic tumor may be present. Note that occasionally, a surgeon will perform an “excisional” biopsy, which effectively accomplishes the same thing as a marginal resection.

Segmental/Wide Resection: An intracompartmental resection. A single piece of bone is resected, including the lesion, adjacent soft tissue, and a cuff of normal bone. Limb salvage is an example.

Radical Resection: The removal of an entire bone, and the excision of the adjacent muscle groups if the tumor is extracompartmental. Amputation is an example.

Fixation

Tissue specimens from bone tumors optimally are received fresh/unfixed in case fresh tissue for ancillary studies, such as cytogenetics, is required. All tissue should be processed in a manner that would allow molecular studies to be undertaken successfully. Decalcification using harsh acidic reagents may be detrimental for nucleic acid-based molecular studies and therefore utilization of EDTA as a decalcifying agent has been advised. Freezing a portion of the sample and/or fixing soft portions of the lesion in buffered formalin is encouraged over EDTA decalcification, for molecular studies.

Tissue Submission for Histologic Evaluation and Genetic/Molecular Studies

One section per centimeter of maximum dimension is usually recommended, although fewer sections are needed for very large tumors, especially if these are homogeneous. Tumors known to be high grade from a previous biopsy do not require as many sections as those that were previously diagnosed as low grade, as documentation of a high-grade component will change stage, prognosis, and treatment in the latter case. All bone tumors that are post-adjuvant therapy (chemotherapy or radiation) have a central slab section entirely mapped and submitted to assess necrosis. Cystic or hemorrhagic areas should be grossly estimated, and the rim entirely submitted. Always, sections should be taken of grossly heterogeneous areas. Occasionally, gross findings can be misleading, and areas that appear to be necrotic may actually be myxoid or edematous. Tumors that have not had pre-adjuvant chemotherapy require at least 1 section per cm and all heterogeneous areas, including those appearing necrotic.

While it has been helpful and often required for clinical trials to have snap frozen tissue, approximately 1 cm$^3$ of fresh tissue stored at minus seventy (-70° C) that can be shipped on dry ice to facilities that perform molecular analysis, most full evaluations of sarcomas can be made on formalin-fixed and EDTA decalcified paraffin-embedded tissue. Adequate tissue should be submitted for conventional light microscopy before tissue has been taken for other analysis. Other than for flow cytometry, microbiology

References
cultures (to send directly to the Microbiology Laboratory from the Operating Room), EM, and cytogenetics, most additional studies for FISH, molecular and copy number/methylation profiling can all be performed on both formalin-fixed and EDTA-fixed tissue (and not on acid decalcified tissue).

Intraoperative Consultation
Most intraoperative assessment is for margins. Histologic classification of bone tumors is sufficiently complex that it is unreasonable to expect a precise classification of these tumors based on an intraoperative consultation. A complete understanding of the surgeon’s treatment algorithm is recommended before rendering a frozen section diagnosis. In the case of primary bone tumors, an intraoperative diagnosis of benign versus malignant will generally guide the immediate decision to curette, excise, or wait for permanent sections, and certain therapeutic options may be lost if the wrong path is pursued. Intraoperative consultation is useful in assessing if “lesional” tissue is present and whether or not this tissue is necrotic, and in constructing a differential diagnosis that can direct the proper triage of tissue for flow cytometry (lymphoma), electron microscopy, and molecular studies/cytogenetics. Tissue triage optimally is performed at the time of frozen section.

References

D. Tumor Site
Given the strong association between the primary anatomic site and outcome, the 8th edition of the AJCC Cancer Staging Manual uses the following site groups for staging purposes:

- Appendicular skeleton, including trunk, skull, and facial bones
- Pelvis
- Spine

This site grouping is reflected by the provision of separate definitions for the primary tumor (T) for each anatomic site.

References
E. Classification of Bone Tumors
The list is derived from the World Health Organization (WHO) classification of soft tissue tumors, 5th edition, edited to include ONLY bone tumors of intermediate (locally aggressive and rarely metastasizing) potential and malignant bone tumors.

Note on atypical cartilaginous tumor/grade 1 chondrosarcoma:
Atypical cartilaginous tumor (ACT) refers to cartilaginous neoplasms demonstrating features of a grade 1 chondrosarcoma and arising in the short and long tubular bones. This terminology should not be used when a pathologist cannot decide on the classification for the cartilaginous neoplasm.

Bone Primary Tyrosine Kinase Fusion Tumors:
While fusions involving the RAS::MAPK pathway are rare among bone tumors, these tumors have driver alterations in genes that encode tyrosine kinases and may respond to therapy targeting NTRK, ALK, BRAF, RET, RAF, FGFR1, or ABL1, etc. Notably, NTRK tumors fused with KANK1 or TPR have been demonstrated to exhibit higher-grade appearance, including spindled and pleomorphic characteristics, accompanied by necrosis and mitoses, leading to unfavorable outcomes. Consequently, it is advisable to conduct comprehensive RNA-based Next-Generation Sequencing (NGS) for fusions, particularly in spindled pleomorphic tumors occurring in individuals under 50 years old, especially those in soft tissue or intraosseous locations. This recommendation is especially pertinent with tumors that have variable ovoid spindled to epithelioid morphology, variable collagenous to myxoid stroma, variable gaping to staghorn vasculature and specifically focal CD34 and/or focal S100 protein, without any staining for SOX10. In these tumors, BRAF, ALK, or panTrk or other immunostain may be identified.

Most Common Molecular/Genetic Findings:
The most common molecular/genetic findings in a subset of intermediate/malignant bone tumors are listed (Table 1).

Table 1: Subset of bone intermediate and malignant tumors with the most common diagnostic molecular/genetic findings.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Genes Involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondrosarcoma</td>
<td>IDH1/IDH2 mutation</td>
</tr>
<tr>
<td>Intraosseous extraskeletal myxoid chondrosarcoma</td>
<td>EWSR1/TAF15::NR4A3 fusion</td>
</tr>
<tr>
<td>Mesenchymal chondrosarcoma</td>
<td>HEY1::NCOA2 fusion</td>
</tr>
<tr>
<td>Secondary chondrosarcoma arising in enchondroma</td>
<td>IDH1/IDH2 mutation</td>
</tr>
<tr>
<td>Secondary chondrosarcoma arising in osteochondroma</td>
<td>EXT1/EXT2 mutation</td>
</tr>
<tr>
<td>Sclerosing epithelioid fibrosarcoma of bone</td>
<td>FUS::CREB3L2 fusion</td>
</tr>
<tr>
<td>Angiomatoid fibrous histiocyntoma of bone/joint</td>
<td>EWSR1::CREB1 or EWSR1::ATF1 alternate</td>
</tr>
<tr>
<td>Tumor Type</td>
<td>Associated Genetic Alterations</td>
</tr>
<tr>
<td>------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Primary malignant giant cell tumor of bone</td>
<td>$H3F3A$ mutation</td>
</tr>
<tr>
<td>Leukemia/Multifocal atypical Langerhans cell histiocytosis</td>
<td>$BRAF$ mutation</td>
</tr>
<tr>
<td>Poorly differentiated chordoma</td>
<td>$SMARCB1$ deletion</td>
</tr>
<tr>
<td>Low-grade central osteosarcoma</td>
<td>$MDM2/CDK4$ amplification</td>
</tr>
<tr>
<td>Parosteal osteosarcoma</td>
<td>$MDM2/CDK4$ amplification</td>
</tr>
<tr>
<td>Rhabdomyosarcoma of bone (adult)</td>
<td>$FUS/EWSR1::TFCP2, MEIS1::NCOA2$</td>
</tr>
<tr>
<td>Ewing sarcoma</td>
<td>$EWSR1::FLI1 (85-90%), EWSR1::ERG (8-10%), others$</td>
</tr>
<tr>
<td>CIC-rearranged sarcoma</td>
<td>$CIC::DX4$</td>
</tr>
<tr>
<td>Round cell sarcoma with $EWSR1::non-ETS$ fusion</td>
<td>$EWSR1::PATZ1, EWSR1::NFATC2, FUS::NFATC2$</td>
</tr>
<tr>
<td>Sarcoma with $BCOR$ genetic alterations</td>
<td>$BCOR::CCNB3$ fusion</td>
</tr>
<tr>
<td>Epithelioid hemangioendothelioma of bone</td>
<td>$WWTR1::CAMTA1$ fusion</td>
</tr>
<tr>
<td>Angiosarcoma of bone</td>
<td>$MYC$ amplification (post-irradiation)</td>
</tr>
<tr>
<td>Tyrosine-kinase fusion tumor</td>
<td>$NTRK1/2/3, ALK, BRAF, etc.$ fusion (various fusion partners)</td>
</tr>
</tbody>
</table>

References


**F. Grading**

The grading of bone tumors is largely driven by the histologic diagnosis, and traditionally grading has been based on the system advocated by Broders, which assesses cellularity and nuclear features/degree of anaplasia. The eighth edition of the AJCC Cancer Staging Manual recommends a 2-tiered system (low vs high-grade) for recording grading. Histologic grading uses a 3-tiered system: Grade 1 is considered low-grade, and Grade 2 and Grade 3 are grouped together as high-grade for biological grading. In bone sarcomas, the histologic subtype often determines the clinical behavior and grade. Therefore, a more pragmatic approach to grading aggressive and malignant primary tumors of bone can be used.

Two bone tumors that are locally aggressive and metastasize infrequently, and thus are usually low-grade, are low-grade central osteosarcoma and parosteal osteosarcoma. Periosteal osteosarcoma is generally regarded as a grade 2 osteosarcoma. Primary bone tumors that are generally high-grade include malignant giant cell tumor, Ewing sarcoma, angiosarcoma, dedifferentiated chondrosarcoma, conventional osteosarcoma, telangiectatic osteosarcoma, small cell osteosarcoma, secondary osteosarcoma, and high-grade surface osteosarcoma.

Grading of conventional chondrosarcoma is based on cellularity, cytologic atypia, and mitotic figures, following the grading system proposed by Evans et al. Grade 1 (low-grade) chondrosarcoma is hypocellular and similar histologically to enchondroma. Grade 2 (intermediate-grade) chondrosarcoma is myxoid and more cellular/atypical than grade 1 chondrosarcoma. Grade 3 (high-grade) chondrosarcoma is hypercellular, pleomorphic, and contains observed mitotic activity.

Mesenchymal chondrosarcoma, fibrosarcoma, leiomyosarcoma, liposarcoma, undifferentiated pleomorphic sarcoma of bone and other “soft tissue-type” sarcomas that rarely occur in bone can be graded according to the French Federation of Cancer Centers Sarcoma Group (FNCLCC) grading system.

Chordomas are locally aggressive lesions with a propensity for metastasis late in their clinical course and are not graded. Adamantinomas tend to have a low-grade clinical course, but this is variable. Fortunately, these are very rare. Other tumors such as periosteal chondrosarcoma (grading
does not predict behavior) or bone angiosarcoma (always considered high-grade behavior) are also not graded. According to the 2020 WHO classification of tumors of bone, adamantinomas are not graded.2,3,6

**Bone Tumor Grades (Most Common)**

**Grade 1 (Low-Grade)**
- Low-grade intramedullary (central) osteosarcoma
- Parosteal osteosarcoma
- Grade I chondrosarcoma
- Clear cell chondrosarcoma

**Grade 2**
- Periosteal osteosarcoma
- Grade II chondrosarcoma

**Grade 3 (High-Grade)**
- Ewing sarcoma
- Most round cell sarcomas
- Sarcoma with BCOR genetic alterations
- CIC-rearranged sarcoma
- Conventional osteosarcoma
- Telangiectatic osteosarcoma
- Mesenchymal chondrosarcoma
- Small cell osteosarcoma
- Secondary osteosarcoma
- High-grade surface osteosarcoma
- Dedifferentiated chondrosarcoma
- Dedifferentiated chordoma
- Poorly differentiated chordoma
- Malignancy in giant cell tumor (primary and secondary malignant giant cell tumor of bone)
- Grade III chondrosarcoma
- Leiomyosarcoma
- Rhabdomyosarcoma
- Undifferentiated pleomorphic sarcoma

**TNM Grading**
The 8th edition of the American Joint Committee on Cancer (AJCC) and International Union Against Cancer (UICC) staging system for bone tumors includes a 3-grade system but effectively collapses into high-grade and low-grade.4,5 Other grading systems in (TNM) are based on differentiation, yet this is not applicable to primary intraosseous sarcomas.

GX  Grade cannot be assessed
G1  Well-differentiated, low-grade
G2  Moderately differentiated, high-grade
G3  Poorly differentiated, high-grade
For purposes of using the AJCC staging system (see note L), 3-grade systems can be converted to a 2-grade (TNM) system as follows: grade 1 = low-grade; grade 2 and grade 3 = high-grade.

References

G. Mitotic Rate
Mitotic rate is determined by counting mitotic figures in the most mitotically active area, away from areas of necrosis, in either 10 consecutive high-power fields (HPF) (use the X40 objective) (1 HPF x 400 = 0.1734 mm²) or in the appropriate number of HPF to encompass 1 mm² based on each individual microscope.

The area of 1 HPF originally used measured 0.1734 mm². However, the area of 1 HPF using most modern microscopes with wider 40x lenses will most likely be higher. Pathologists are encouraged to determine the field area of their 40x lenses and divide 0.1734 by the obtained field area to obtain a conversion factor. The number of mitotic figures in 10 HPF multiplied by the obtained conversion factor and rounded to the nearest whole number should be used for reporting purposes.

An important change in the 5th Edition of the WHO Classification of Tumours series¹ is the conversion of mitotic count from the traditional denominator of 10 HPF to a defined area expressed in 1 mm², as an attempt to standardize the area used for mitotic count. Table 2 demonstrates the approximate number of fields required to encompass 1 mm² based on the field diameter and its corresponding area.

<table>
<thead>
<tr>
<th>Field diameter (mm)</th>
<th>Area (mm²)</th>
<th>Approximate number of fields per 1 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.40</td>
<td>0.126</td>
<td>8</td>
</tr>
<tr>
<td>0.41</td>
<td>0.132</td>
<td>8</td>
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<tr>
<td>0.42</td>
<td>0.138</td>
<td>7</td>
</tr>
<tr>
<td>0.43</td>
<td>0.145</td>
<td>7</td>
</tr>
</tbody>
</table>
References

H. Response to Chemotherapy/Radiation Therapy Effect
It is essential to estimate neoadjuvant treatment effect in primary Ewing sarcoma and osteosarcoma of bone, as these have been shown to have prognostic significance1,2,3,4,5,6. An entire representative slice of the tumor taken through the long axis should be mapped using a grid pattern diagram, photocopy, or radiologic film to indicate the site for each tumor block. In addition, the remainder of the neoplasm should be sampled at the rate of 1 section per centimeter. Areas of soft tissue extension and the interface of tumor with normal tissue should also be sampled. The sum of all viable areas measured microscopically is divided by the total cross-sectional area occupied by tumor to arrive at a percentage. One way to do so is to estimate the percent treatment effect (necrosis/fibrosis/hemorrhage/histiocytic response) on each slide of the grid slab (greatest surface area of tumor in the longest axis) and put these measurements into
an Excel spreadsheet and assess the average necrosis of all slides examined. This yields a finding that is compatible with treatment assessment. Prognostically significant therapy response in osteosarcoma, according to most series, is defined >90%, with those tumors showing >90% therapy response associated with a favorable prognosis.²³⁴ There are 2 protocols to assess response to therapy in Ewing sarcoma. Response can be assessed in the same manner as osteosarcoma or by the system of Picci, which is expressed as grade I (macroscopic viable tumor), grade II (microscopic viable tumor), or grade III (no viable tumor).⁵⁶

Histologic Classification of Treated Lesions

Due to extensive treatment effect, such as necrosis, fibrosis, and chemotherapy-induced and radiation-induced pleomorphism, it may not be possible to classify some lesions that were either never biopsied or where the biopsy was insufficient for a precise diagnosis. In problematic cases, the grade of the pretreatment specimen (i.e., biopsy, if available) should take precedence.

References


I. Lymphatic and/or Vascular Invasion

Lymphatic or vascular invasion (LVI) indicates whether microscopic lymphatic or vascular invasion is identified. LVI includes lymphatic invasion or vascular invasion or both. By AJCC/UICC convention, LVI does not affect the T category indicating local extent of tumor unless specifically included in the definition of a T category.

J. Margins

It has been recommended that for all margins located less than 2 cm, the distance of the tumor from the margin be reported in centimeters.¹ However, there is a lack of agreement on this issue.² We recommend specifying the closest margin only and optionally the location of all margins less than 0.2 cm.³ Margins from bone tumors should be taken as perpendicular (radial) margins, if possible. If the tumor is located more than 2 cm from the bone margin, the marrow can be scooped out and submitted as a margin.
References


K. Regional Lymph Nodes

Regional lymph node metastasis is extremely rare in adult bone sarcomas. Nodes are not sampled routinely, and it is not necessary to exhaustively search for nodes. When no lymph nodes are resected, the pathologic ‘N’ category is not assigned (pNX is not used for bone tumors). When present, regional lymph node metastasis has prognostic importance and should be reported. Patients whose nodal status is not determined to be positive for tumor, either clinically or pathologically, should be designated as N0.

References


L. pTNM Classification

The 8th edition TNM staging system for bone tumors of the AJCC and the UICC is recommended. Anatomical staging using the AJCC system 8th ed. is considered clinically relevant only for the specific malignant entities listed in the World Health Organization classification 5th ed. This includes malignant chondrogenic tumors, osteogenic tumors, fibrogenic tumors, osteoclastic giant cell-rich tumors, notochordal tumors, myogenic tumors, lipogenic tumors, undifferentiated small round cell sarcomas and other mesenchymal tumors arising in bone. Locally aggressive entities such as synovial chondromatosis, osteoblastoma, giant cell tumor of bone, epithelioid hemangioma, pseudomyogenic hemangioma, and desmoplastic fibroma may be reported using this protocol but are not staged. Site groups for bone sarcomas are the following: appendicular skeleton, including trunk, skull and facial bones, pelvis, and spine. Pathologic staging includes pathologic data obtained from examination of a resected specimen sufficient to evaluate the highest T category, histopathologic type and grade, regional lymph nodes as appropriate, or distant metastasis. Because regional lymph node involvement from bone tumors is rare, the pathologic stage grouping includes any of the following combinations: pT pG pN pM, or pT pG cN cM, or cT cN pM. Biological grade should be assigned to all bone sarcomas, and based on published outcomes data, the current staging system accommodates a two-tiered (low- vs high-grade) system for recording grade. Histologic grading (G) uses a three-tiered system: G1 is considered low-grade, and G2 and G3 are grouped together as high-grade for biological grading.
TNM Descriptors
For identification of special cases of TNM or pTNM classifications, the "m" suffix and the "y" and "r" prefixes are used. Although they do not affect the stage grouping, they indicate cases needing separate analysis.

The “m” suffix indicates the presence of multiple primary tumors in a single site and is recorded in parentheses: pT(m)NM.

The “y” prefix indicates those cases in which classification is performed during or following initial multimodality therapy (i.e., neoadjuvant chemotherapy, radiation therapy, or both chemotherapy and radiation therapy). The cTNM or pTNM category is identified by a “y” prefix. The ycTNM or ypTNM categorizes the extent of tumor actually present at the time of that examination. The “y” categorization is not an estimate of tumor prior to multimodality therapy (i.e., before initiation of neoadjuvant therapy).

The “r” prefix indicates a recurrent tumor when staged after a documented disease-free interval and is identified by the “r” prefix: rTNM.

T Category Considerations (Figures 2 and 3)
Spine segments for staging:

Figure 2. Spine segments for staging. Used with permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois. The original source for this material is the AJCC Cancer Staging Manual (2017) published by Springer Science and Business Media LLC, www.springerlink.com.
Figure 3. Pelvic segments for staging. Used with permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois. The original source for this material is the *AJCC Cancer Staging Manual* (2017) published by Springer Science and Business Media LLC, [www.springerlink.com](http://www.springerlink.com)

N Category Considerations
Because of the rarity of lymph node involvement in sarcomas, the designation NX may not be appropriate and could be considered N0 if no clinical involvement is evident.

References