Protocol for the Examination of Specimens from Patients with Primary Tumors of Bone

Protocol applies to primary malignant bone tumors including chondrogenic tumors, osteogenic tumors, fibrogenic tumors, osteoclastic giant cell rich tumors, notochordal tumors, vascular tumors, myogenic tumors, and lipogenic tumors. This protocol does not apply to plasma cell neoplasms, lymphoma, pediatric Ewing sarcoma, and soft tissue sarcoma.

Based on:

AJCC/UICC TNM, 8th edition
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Revision History:

None

Summary of Changes:

This protocol is revised to the 8th edition of the AJCC Cancer Staging Manual and the current version of the CAP Cancer Protocol Bone 4.0.0.0.

Procedures Covered in this Protocol:

- Biopsy
  - Core needle biopsy
  - Curettage
  - Excisional biopsy
- Resection
  - Intralesional resection
  - Marginal resection
  - Segmental/wide resection
  - Radical resection

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The purpose of the Protocols is to support Laboratory Personnel engaged in the macroscopic examination of cancer resection specimens. The Protocols are based on specified relevant source documents, drafted by pathologists' assistant experts, and supported by information provided by the College of American Pathologists (CAP) and the American Joint Committee on Cancer (AJCC). These Protocols are intended to serve patients by ensuring that the macroscopic examination of cancer resection specimens is compliant with CAP Cancer Protocols, the AJCC Cancer Staging Manual, and provide optimization of the pre-analytic steps necessary to promote appropriate molecular studies.

The AAPA cautions that the use of the Protocols in practice may require the use of additional considerations that are beyond the scope of the Protocols. The AAPA does not offer medical advice or diagnoses, or engage in the practice of medicine. The information provided in the Protocols is not intended or implied to be a substitute for the Laboratory Personnel's own training, professional medical opinion, diagnosis, or treatment advice. All content, including text, graphics, images and information contained in the Protocols are for the above stated purposes only. Laboratory Personnel are encouraged to confirm any information provided in these Protocols with other sources. The inclusion of a product name, organization, or service in an AAPA publication, including without limitation the Protocols, should not be construed as an endorsement of such product, organization, or service, nor is failure to include the name of a product, organization or service to be construed as disapproval.

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Ambiguous Terminology:

Marginal resection and excisional biopsy both refer to removal of the tumor and its pseudocapsule with a relatively small amount of adjacent tissue.

Molecular Considerations:

Tissue specimens from bone tumors optimally are received fresh / unfixed because of the importance of ancillary studies.

Once sufficient tissue is reserved for histologic examination, a 1 cm^3 piece of fresh tumor should be cut into 0.2 cm fragments and snap frozen. The frozen tissue can be stored at -70 degrees C and shipped on dry ice if necessary for molecular analysis. Analysis may include a variety of molecular assays for tumor-specific molecular translocations that help classify bone tumors.

For small specimens, such as core biopsies, less tissue is acceptable. A frozen section residuum, previously used for evaluation of specimen adequacy and viability, can be saved frozen at -70 degrees C and used for this purpose.

FISH and RT-PCR studies are used to evaluate tumor-defining translocations for Ewing sarcoma family of tumors. Due to increased sensitivity, snap frozen tissue is preferred, and every effort should be made to procure a sample.

Once adequate tissue has been submitted for light microscopy and frozen for potential molecular testing, save tissue for cytogenetic analysis and electron microscopy, or flow cytometry if lymphoma is suspected. Chromosomal analysis has gained increasing importance in classification, prognosis, and treatment choice for a range of sarcomas.

Cytogenetic Considerations:

Submit fresh tumor in alpha MEM or other suitable transport media for cytogenetic analysis.

Electron Microscopy:

Submit 1 mm cubes of fresh tumor in 2% glutaraldehyde or other suitable fixative for electron microscopy.

Immunohistochemistry Considerations:

Expression of p16 by untreated osteosarcoma has been found to correlate with percentage of necrosis. Use of p16 in pretreatment biopsies may predict which osteosarcomas will have a good response to standard neoadjuvant chemotherapy.

The macroscopic description should disclose the initial handling of the fresh specimen so that the pathologist is aware of tissue availability for ancillary studies. The macroscopic description should provide the fixative used. 10% neutral buffered formalin is the preferred fixative. It is recommended that the duration of fixation be provided. The use of decalcifying agents should be provided as well.

PROCEDURES AND GENERAL ANATOMIC CONSIDERATIONS:

- **Procedures Covered by this Protocol:**

  - Core needle biopsy
  - Curettage / Intralesional resection
  - Excisional biopsy / marginal resection
  - Wide / segmental resection
  - Radical resection

- **Specimen Size and Extent of Resection:**

  Biopsy (Needle, Curettage, Excisional):

  - Measure the specimen in three dimensions.
  - Core needle biopsies are used to extract a tissue core from the innermost part of the bone and can obtain sufficient material for special studies and morphologic diagnosis.
  - Excisional biopsy may not include an adequate margin of normal tissue, even with an operative impression of total gross removal.

- **Explanatory Notes:**

  Radiographic imaging plays an especially critical role in the diagnosis of bone tumors. In cases of nonexcisional biopsy (e.g., core biopsy, currettage), the tumor size cannot be determined on pathologic grounds; therefore, imaging data (computed tomography, magnetic resonance imaging, and radiographs) may be used instead.

  Resection:

  The extent of resection specimens may be intralesional, marginal, segmental / wide, or radical.

  - For all types of resections, orientation of the specimen and application of ink prior to sectioning are highly recommended.
  - Curettage / Intralesional resection: scraping of the bone with partial debulking, leaving tumor behind.
  - Excisional biopsy or marginal resection: removal of the tumor and its pseudocapsule with a small amount of adjacent tissue; there is no macroscopic tumor at the margin.
  - Wide resection / segmental resection: a piece of bone is resected, including the lesion and cuff of normal bone.
  - Radical resection: removal of an entire bone (e.g., above the knee amputation), or the excision of the adjacent muscle groups if the tumor is extracompartmental.
  - Document and measure each structure present, including length and maximum circumference of limbs.
  - If specimen is a portion of bone, describe which anatomic landmarks of that bone are included (e.g., epiphysis, diaphysis, apophysis, etc.).
  - If specimen is an amputation or an en-bloc resection, measure and describe all structures attached.

  Full representative mapping of the specimen is recommended (See Tumor Mapping Section).

- **Specimen Integrity and Adequacy:**

  As previously stated, bone tumor specimens should be received in the fresh state due to the importance of ancillary studies, such as cytogenetic analysis, which require fresh tissue.

  Provide an assessment of the specimen integrity, identifying and describing any defects or disruptions.

  Statements should describe with as much clarity as possible the anatomic location of the defect(s) or disruption(s), including assessment of the relationship(s) to the tumor or margin(s). If defects or disruptions involve the tumor or margins and serve to hinder assessment of the final surgical margin(s), this must be stated. Consultation with the surgeon for clarification should be considered, as well as differentially inking the margins in areas affected by defects or disruptions, as this may aid in creating a post-surgical treatment plan.

TUMOR ("T" of TNM)

- **Tumor Size:**
  - Provide measurement in three dimensions in cm for biopsy specimens.
  - Provide measurement in three dimensions in cm or length and maximum circumference of limbs.
  - Specify if the lesion is continuous, multifocal or discontinuous from the primary site (skip metastasis).
  - For Ewing sarcoma, tumor size of 8 cm or less portends a better prognosis than a tumor size greater than 8 cm.
  - For osteosarcoma, tumor size less of 9 cm or less suggests a better prognosis than tumor size greater than 9 cm.

- **Tumor Site(s):** *(Figure 1)*

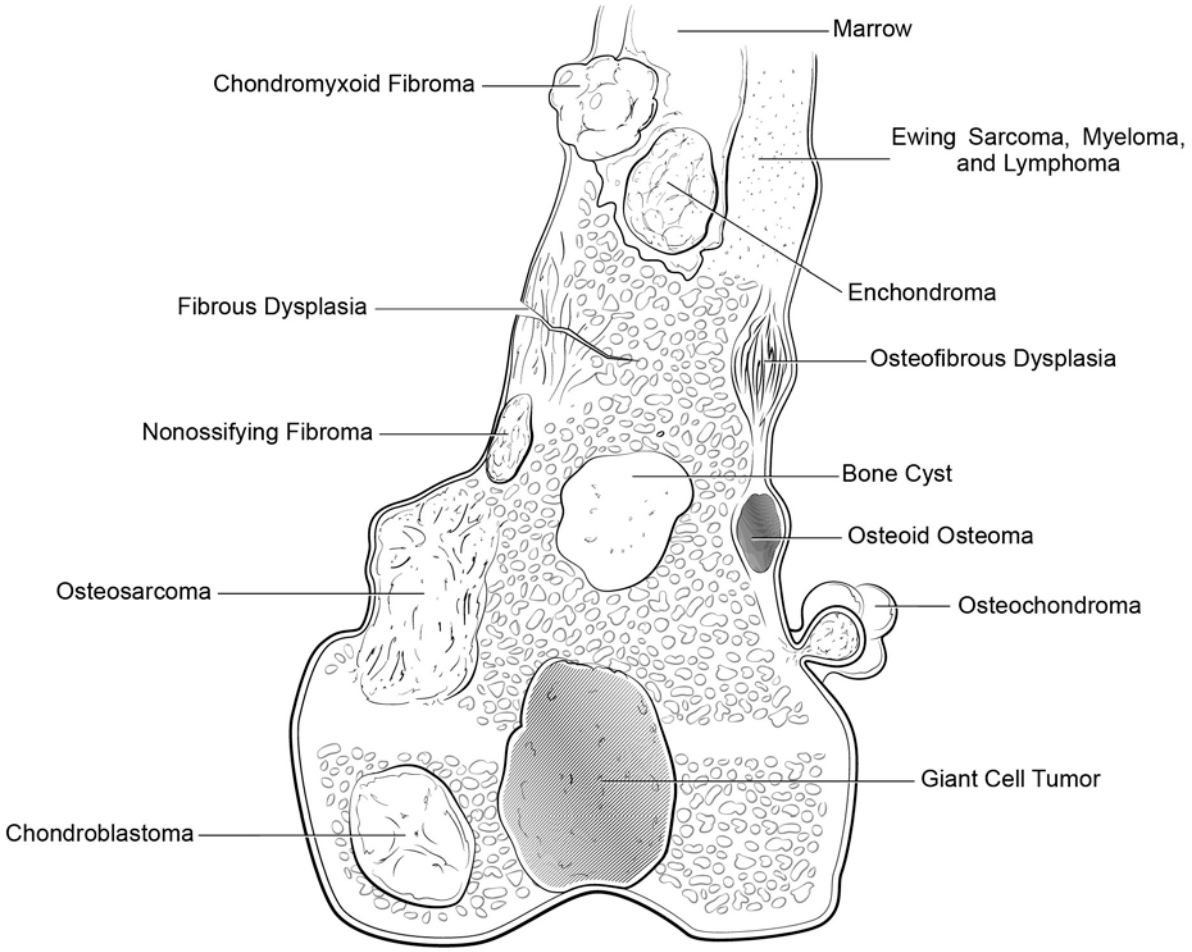

Figure 1: Bone Tumor Common Locations

- 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

    **There are separate definitions for the primary tumor (T) for each anatomic site. Patients with tumors of the extremities have a better prognosis than those with tumors arising in the pelvis and spine.*

- Specify the tumor location in the bone, as the location of a bone tumor is a prognostic factor: *(Figure 2)*
    - Epiphysis or apophysis
    - Diaphysis
    - Metaphysis
    - Cortex
    - Medullary cavity
    - Periosteum (surface)
    - Tumor involves joint
    - Tumor extension into soft tissue

- If the tumor extends beyond a single location, provide all involved locations and, if possible, provide the location of the epicenter of the tumor.

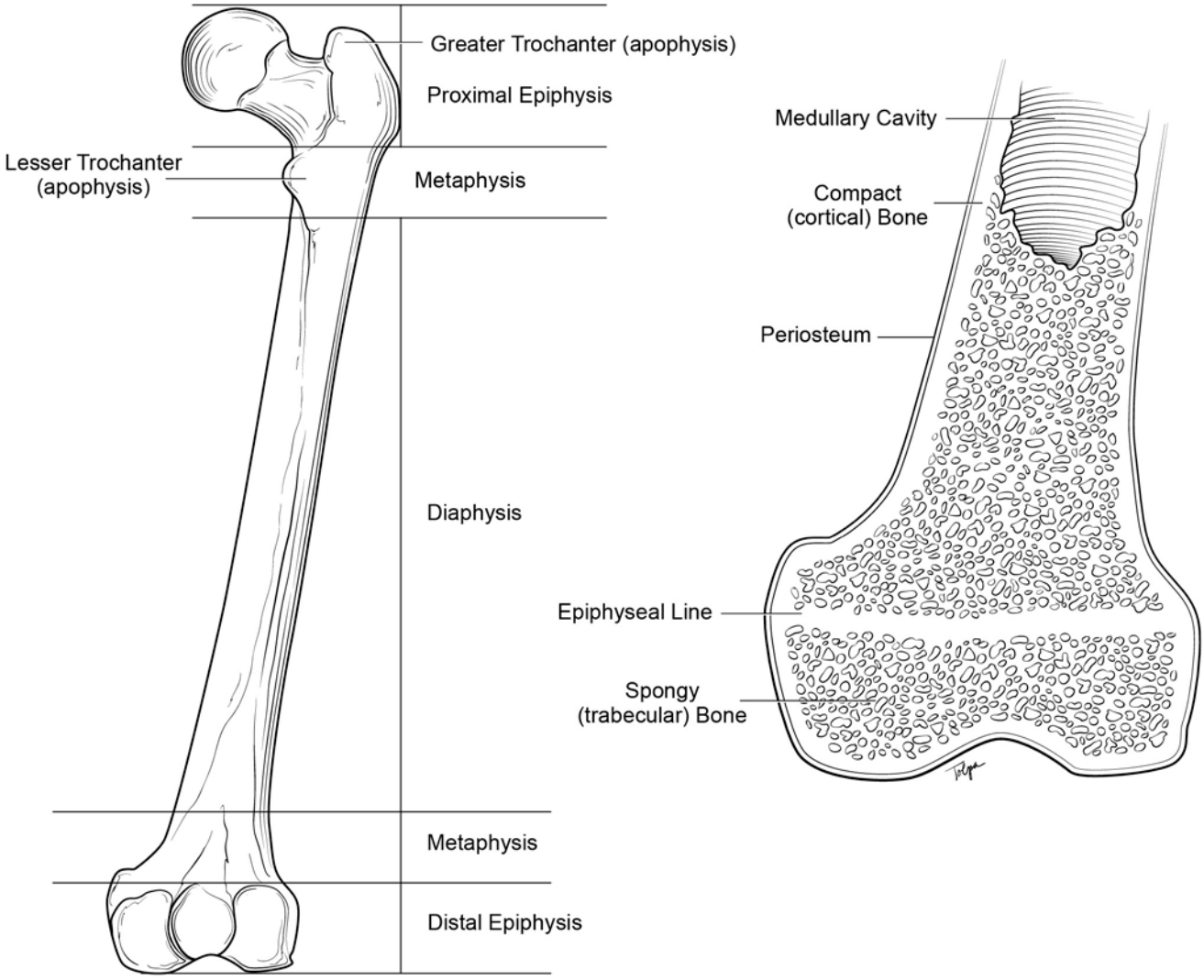

Figure 2: Anatomic Landmarks for Tumor Diagnosis in Long Bones

- **Tumor Depth of Invasion and Relationship to Attached Organs / Structures:**

  - Specify if the tumor erodes through the cortex and extends into soft tissue or surrounding structures.
  - Specify if there is extension through the epiphyseal plate; extension into or across the joint space.
  - Specify if vascular involvement is identified macroscopically.
  - Specify if skip metastases are identified macroscopically.
  - Biopsy of bone tumors can complete the clinical staging process, if necessary.
  - The biopsy site should be included in the tumor resection specimen.
  - Pathologic staging is completed by gross exam of resection specimen.
  - Imaging should be referenced to correlate with clinical staging.

Definition of Primary Tumor (pT)

Appendicular Skeleton, Trunk, Skull, and Facial Bones

pT Category	pT Criteria
pTX	Primary tumor cannot be assessed
pT0	No evidence of primary tumor
pT1	Tumor ≤ 8 cm in greatest dimension
pT2	Tumor > 8 cm in greatest dimension
pT3	Discontinuous tumors in the primary bone site

Spine

pT Category	pT Criteria
pTX	Primary tumor cannot be assessed
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

Pelvis

pT Category	pT Criteria
pTX	Primary tumor cannot be assessed
pT0	No evidence of primary tumor
pT1	Tumor confined to one pelvic segment with no extraosseous extension
pT1a	Tumor ≤ 8 cm in greatest dimension
pT1b	Tumor > 8 cm in greatest dimension
pT2	Tumor confined to one pelvic segment with extraosseous extension or two segments without extraosseous extension
pT2a	Tumor ≤ 8 cm in greatest dimension
pT2b	Tumor > 8 cm in greatest dimension
pT3	Tumor spanning two pelvic segments with extraosseous extension
pT3a	Tumor ≤ 8 cm in greatest dimension
pT3b	Tumor > 8 cm in greatest dimension
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

***TNM Descriptors**:*

The prefixes and suffixes listed here are for the identification of special cases and do not affect the stage groupings. These 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). 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.

The "a" prefix designates the stage determined at time of autopsy: aTNM.

Residual Tumor (R) Category

The absence or presence of residual tumor at the primary tumor site after treatment is denoted by the symbol R. The R categories for the primary tumor site are as follows:

R	R Definition
RX	Presence of residual tumor cannot be assessed
R0	No residual tumor
R1	Microscopic residual tumor
R2	Macroscopic residual tumor at the primary cancer site or regional nodal sites

For the pathologist, the R classification is relevant to the status of the surgical resection specimen. Tumor involving the resection margin may be classified as macroscopic or microscopic according to the findings at the specimen margin(s). To the surgeon this indicates the status of surgical excision completeness.

- **Recommendations for tissue submission:**
  (Clinical history is important as treatment status guides sampling.)
  (Imaging information is essential for bone tumor diagnosis.)

  - Soft tissue and bone margin
  - Intramedullary component of the lesion
  - Extension of the tumor into the soft tissue, vessels, and nerves
    - Sections of all normal structures (e.g., major vessels and nerve trunks)
  - Tumor penetrating through the cortex
  - Tumor in relation to the normal bone
  - Involvement of the tumor to an articular surface or joint space
  - Submit one section per centimeter of maximum dimension of tumor, unless it is large and homogeneous.
  - Tumors previously diagnosed as low grade require more sections than those previously diagnosed as high grade.
  - Tumors with greater heterogeneity should be sampled more thoroughly, and there is no need to submit more than one section of necrotic tumor (always with a transition to viable tumor), with the exception of chemotherapy effect on treated tumors.
  - Occasionally, macroscopic pathology can be misleading, and areas that appear to be macroscopically necrotic may actually be myxoid or edematous. When this happens, additional sections should be submitted for histologic examination.
  - Because percent necrosis must be provided, state which sections include necrotic areas so that microscopic confirmation can be established, lending support to the macroscopic estimate of necrosis.
  - In some instances, (where heterogeneity or other features create uncertainty with regard to percent of macroscopic necrosis) it may be appropriate to provide the percent of necrosis in the tissue sections submitted for microscopic evaluation as this allows the pathologist to confirm the macroscopic accuracy. For instance, consider submitting a single section for microscopic examination and, in the summary of sections, state the percent of necrosis in the section submitted (e.g., cassette x: section demonstrating viable tumor and necrotic tumor (50% viable; 50% necrotic).

  Note the presence and / or absence of necrosis, and bone or cartilage formation. When a patient has received neoadjuvant chemotherapy, it is important to estimate the percentage of tumor necrosis for prognostic purposes. Prognostically significant therapy response in osteosarcoma is defined at 90%, with those tumors showing 90% therapy response associated with a favorable prognosis.

  Osteosarcoma and Ewing Sarcoma:

  An entire representative slice through the long axis of the bone should be mapped using a grid pattern. Refer to the tumor mapping section below.

- **Specimen Processing and Tumor Mapping Guidelines:** *(Figure 3)*

  Specimen Processing:

  - Radiograph the intact specimen to determine in which plane to cut the bone slab.
  - Apply ink to the external surface and submit soft tissue margins.
  - Freeze the specimen overnight in a -70-degree freezer or fix in 10% neutral buffered formalin.
  - Incise the soft tissue in a plane that will demonstrate the greatest extent of the tumor.
  - Bisect the specimen with a band saw.
  - Cut a 0.5 cm thick parallel section through the bone.

  Tumor Mapping:

  - Photograph, radiograph, or photocopy (in a plastic bag) the bone slab.
  - This central full face of the specimen and lesion is mapped by drawing a grid on the radiograph or photograph; the entire cross-section is mapped and submitted for microscopic examination and estimation of percentage of tumor necrosis.
  - After formalin fixation and decalcification, sections are taken to demonstrate the tumor, relationship to adjacent normal bone, invasion of contiguous structures, and margins.
  - The location of sections taken is indicated on the diagram of the specimen.
  - The entire cross-section is sampled and correlated with the radiograph.

- **Explanatory Notes:**

  Bone dust can create artifacts that may be difficult to interpret. Sections should be oriented so that the portion cut by the histotechnologist will be opposite the side cut by the saw such as applying ink to one side and indicating the appropriate side to be sectioned. Alternatively, bone dust can be cleaned off with a brush or by rinsing in saline.

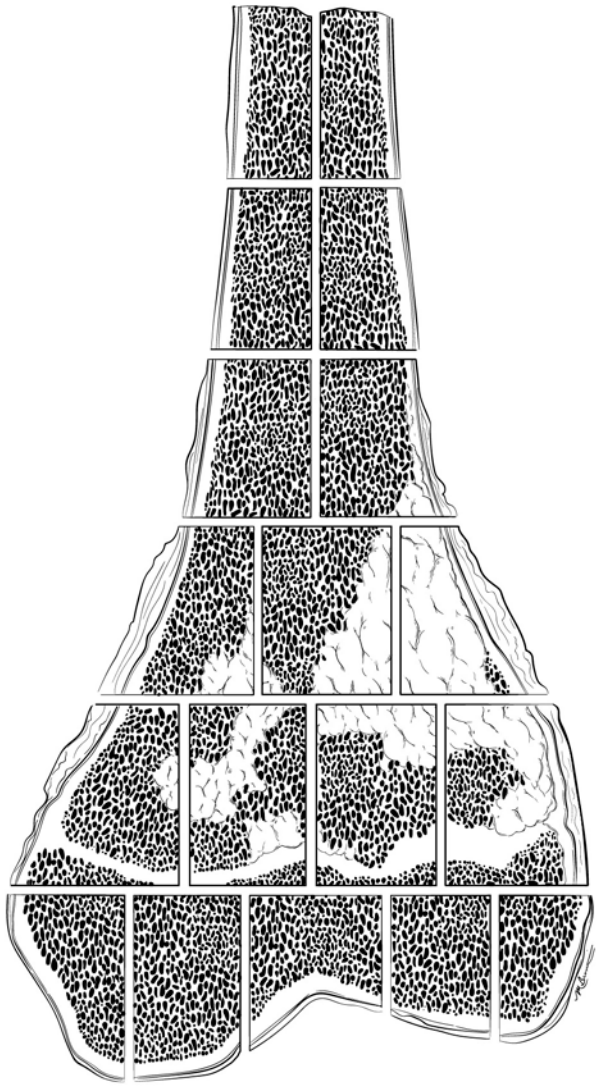

Figure 3: Bone tumor map/grid demonstrating histologic sections

- **Margins:**

  - Distance of tumor to all margins (bone, soft tissue, marrow, skin) should be measured and specified.
  - State if margins are macroscopically involved or uninvolved by tumor.
  - Specify the location of all margins < 2 cm and submit perpendicular sections if possible.
  - If the tumor is > 2 cm from the margin, the marrow can be scooped out or scraped and submitted as a margin.
  - State if the neurovascular bundle at the margin is involved or uninvolved by tumor.

LYMPH NODES ("N" of TNM)

- **Lymph Nodes:** (if applicable)

Definition of Regional Lymph Node (pN)

pN Category	pN Criteria
pNX	Regional lymph nodes cannot be assessed. Because of the rarity of lymph node involvement in bone sarcomas, the designation pNX may not be appropriate, and cases should be considered pN0 unless clinical node involvement clearly is evident.
pN0	No regional lymph node metastasis
pN1	Regional lymph node metastasis

Lymph node involvement is rare in bone sarcomas.

Regional and Nonregional Lymph Node Submission:

- Count lymph nodes identified.
- Specify site of lymph nodes identified (regional, nonregional).
- Measure lymph nodes in three dimensions.
- Describe the cut surface of the identified lymph nodes.
- Submit all lymph nodes for microscopic examination.
    - Submit small lymph nodes in toto.
    - Serially section and entirely submit larger macroscopically negative lymph nodes.
    - Representative sections from macroscopically positive nodes are adequate.
- If possible, submit lymph node sections so that the long axis of the lymph node is demonstrated.
- Take steps to ensure that an accurate lymph node count can be rendered.

METASTASIS ("M" of TNM)

- **Metastasis:**

Definition of Distant Metastasis (pM) (required only if confirmed pathologically)

pM Category	pM Criteria
M0	No distant metastasis
pM1	Distant metastasis
pM1a	Lung
pM1b	Metastasis involving other distant sites other than lung

Metastatic Sites:

- A metastatic site includes any site beyond the regional lymph nodes of the primary site.
- Pulmonary metastases are the most frequent site for all bone sarcomas.
  - Patients with solitary lung metastasis have a better prognosis than those with multiple lung metastases. Consequently, it is important to document the number of lung metastases.
- Extra-pulmonary metastases occur infrequently and may include secondary bone metastases which suggest a worse prognosis.

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