Protocol for the Examination of Specimens from Patients with Retinoblastoma

Protocol applies to retinoblastoma only.

Based on:

AJCC/UICC TNM, 8th edition
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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 Retinoblastoma 4.0.0.0.

Procedures Covered in this Protocol:

- Enucleation
- Partial exenteration
- Complete exenteration

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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.

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Molecular Considerations:

Identification of *RB1* mutations and other genetic studies in tumor tissue are difficult with formalin-fixed tissue. Consequently, neoplastic tissue for genetic studies should be harvested prior to fixation.

Forty percent of retinoblastomas are germinal, and patients may have a family history. The patient may be at risk of passing the mutated *RB1* gene on to the next generation.

Definition of Heritable Trait (H)

H Category	H Criteria
HX	Unknown or insufficient evidence of a constitutional *RB1* gene mutation
H0	Normal *RB1* alleles in blood tested with demonstrated high-sensitivity assays
H1	Bilateral retinoblastoma, retinoblastoma with an intracranial primitive neuroectodermal tumor (i.e., trilateral retinoblastoma), patient with family history of retinoblastoma, or molecular definition of a constitutional *RB1* gene mutation

Specimen Handling for molecular testing (these directions may need to be provided to the surgeon prior to surgery if tumor samples are collected intraoperatively):

The surgical optic nerve margin should be obtained prior to opening the globe to prevent contamination with artifactual clumps of tumor cells (“floaters”).

The margin should be applied with ink, and the optic nerve stump cut approximately 2 mm from the sclera with a sharp razor. The optic nerve should then be placed separate from the globe into a jar of 10% neutral buffered formalin.

A sample of tumor is obtained by opening a small sclero-choroidal window adjacent to the tumor near the equator with a 6 to 8 mm corneal trephine. Tumor tissue should be gently removed with forceps and scissors through the established opening. If the specimen is received with a sclero-choroidal window, this must be stated in the macroscopic description.

> *Fresh tumor may then be placed in MEM-transport media or other suitable transport media for cytogenetic analysis.*
>
> *Snap-frozen tumor for molecular studies may also be desired if enough tumor is available.*

It is best to leave a hinge on one side of the scleral flap, so it can be closed with 1 or 2 suture(s) following the removal of the tumor sample. This is done to maintain the overall spherical architecture of the specimen during fixation.

The globe should be placed in a second jar of formalin and be allowed to fix for at least 24 to 48 hours.

Immunohistochemistry Considerations:

Retinoblastoma is positive for S100, NSE, synaptophysin, and GFAP. This tumor also has a high Ki-67 proliferation index.

These immunohistochemistry tests can be performed on formalin fixed paraffin embedded tissue sections. 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 as well.

PROCEDURES AND GENERAL ANATOMIC CONSIDERATIONS:

- **Procedures Covered by this Protocol:**

  - Enucleation: Removal of the globe and portion of optic nerve leaving the eye muscles and remaining orbital contents intact.
  - Partial exenteration: Removal of globe and partial removal of surrounding orbital soft tissue.
  - Complete exenteration: Removal of the globe and all the orbital contents including surrounding orbital soft tissue, musculature, some or all of the eyelids, and part of the bony orbit.
  - Other (specify)

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

  - Enucleation:
    - Anteroposterior diameter in mm
    - Horizontal diameter in mm
    - Vertical diameter in mm
    - Optic Nerve Segment– Length and diameter in mm

  - Exenteration:
    - Provide three dimensions of specimen and attached structures in cm.

- **Specimen Laterality:**

  - Specify right or left.

- **Globe Orientation:** *(Figure 1)*

  The orientation of a globe may be determined by identifying extraocular muscle insertions, optic nerve, and other landmarks. The terms *temporal* and *nasal* are generally used in place of *lateral* and *medial* with reference to ocular anatomy.

  - The inferior oblique muscle insertion is located temporal (lateral) to the optic nerve on the sclera.
  - The inferior oblique fibers travel inferonasally from its insertion.
  - The long posterior ciliary artery is seen as a blue-gray line in the sclera on either side of the optic nerve and marks the horizontal meridian of the globe.

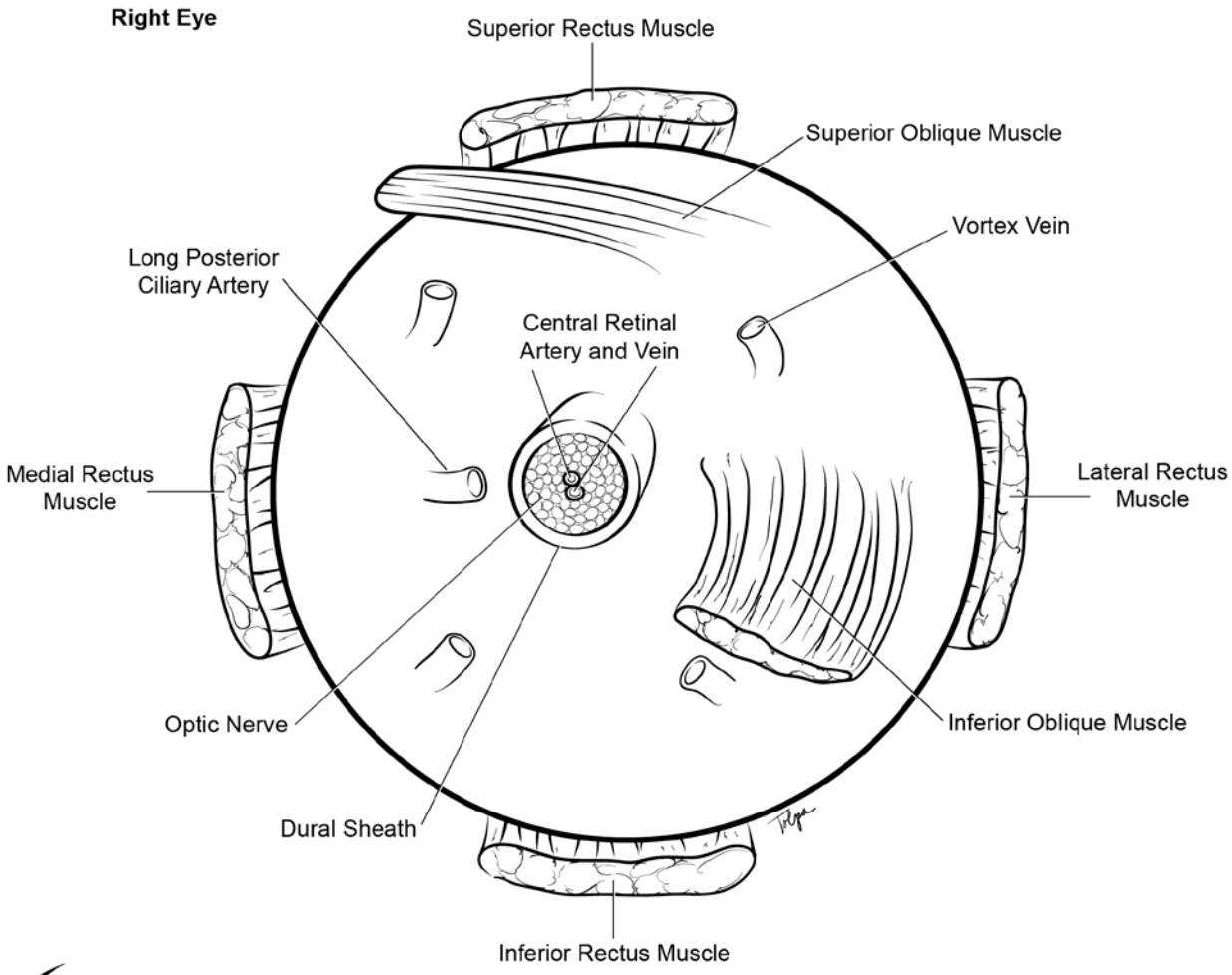

Figure 1: Anatomic Landmarks of the Posterior Aspect of the Globe

- **Specimen Integrity and Adequacy:**

  Any defects or irregularity of the specimen should be described with relation to anatomic landmarks. This is particularly important if these defects affect the surgical margins, most importantly the optic nerve margin. The surgeon may need to be consulted in this case to clarify surgical defects or intraoperative tumor sampling.

  - Inspect the specimen for prior intraoperative tumor sampling.
    - Prior intraoperative tumor sampling can be identified by a small, sclero-choroidal window adjacent to the tumor near the equator. A sutured hinge will be identified on one side of the scleral flap.
    - The optic nerve surgical margin may be received in a separate container, as this margin is removed prior to intraoperative tumor sampling.

  - Specify if the globe is received intact (no intraoperative tumor sampling).

TUMOR ("T" of TNM)

- **Tumor Size:**

  - Tumor Basal Area on Transillumination: *
    - Anterior-Posterior length in mm
    - Transverse length in mm
  - Tumor Size After Sectioning:
    - Provide the greatest basal diameter and thickness in mm.
      - Provide measurement of base at cut edge in mm.
    - Provide the greatest thickness in mm.
      - Provide measurement of thickness at cut edge in mm.
    - Cannot be determined.

  *In most cases, transillumination may not be definitive due to the diffuse nature of the tumor.

- **Tumor Site(s):**

  - Specify location of tumor by macroscopic examination / transillumination:
    - Superotemporal quadrant of globe
    - Superonasal quadrant of globe
    - Inferotemporal quadrant of globe
    - Inferonasal quadrant of globe
    - Anterior chamber
    - Between___ and ___ o'clock
    - Other (specify)
    - Cannot be determined
  - Specify tumor location after sectioning:
    - Superonasal
    - Inferonasal
    - Superotemporal
    - Inferotemporal
    - Provide the distance from the anterior edge of tumor to the limbus at the cut edge in mm.
    - Provide the distance of the posterior margin of tumor base from the edge of optic disc in mm.
    - Cannot be determined

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

  Local extension anteriorly can result in soft tissue involvement of the face or a mass protruding from between the lids. Posterior extension results in retinoblastoma extending into the orbit, paranasal sinuses, and/or brain.

- Indicate tumor involvement of other ocular structures including:
    - Cornea
    - Anterior chamber
    - Iris
    - Angle
    - Lens
    - Ciliary body
    - Vitreous
    - Retina
    - Sub-retinal space
    - Sub-retinal pigment epithelial space
    - Optic nerve head
    - Choroid, minimal (solid tumor nest less than 3 mm in maximum diameter [width or thickness])
    - Choroid, massive (solid tumor nest 3 mm or more in maximum diameter [width or thickness])
    - Sclera
    - Vortex vein
    - Orbit
    - Other (specify)
    - Cannot be determined

- Indicate Extent of Optic Nerve Invasion:
    - Anterior to lamina cribrosa
    - At lamina cribrosa
    - Posterior to lamina cribrosa but not to end of nerve
    - To cut end of optic nerve (determined microscopically)
    - No invasion of optic nerve (determined microscopically)
    - Cannot be determined

- Growth Pattern:
    - Endophytic - growth from inner retinal surface into the vitreous cavity
    - Exophytic - growth primarily from outer surface of the retina into the subretinal space toward the choroid
    - Combined endophytic/exophytic - exhibits features of both endophytic and exophytic growth
    - Diffuse - infiltrating tumors grow laterally within the retina without significant thickening
    - Anterior diffuse – a rare variant of retinoblastoma with seeding of the vitreous base and anterior chamber
    - Cannot be determined

Pathological Classification

Definition of Primary Tumor (pT)

pT Category	pT Criteria
pTX	Unknown evidence of intraocular tumor
pT0	No evidence of intraocular tumor
pT1	Intraocular tumor(s) without any local invasion, focal choroidal invasion, or pre- or intralaminar involvement of the optic nerve head
pT2	Intraocular tumor(s) with local invasion
pT2a	Concomitant focal choroidal invasion and pre- or intralaminar involvement of the optic nerve head
pT2b	Tumor invasion of stroma of iris and/or trabecular meshwork and/or Schlemm's canal
pT3	Intraocular tumor(s) with significant local invasion
pT3a	Massive choroidal invasion (>3 mm in largest diameter, or multiple foci of focal choroidal involvement totaling >3 mm, or any full-thickness choroidal involvement)
pT3b	Retrolaminar invasion of the optic nerve head, not involving the transected end of the optic nerve
pT3c	Any partial-thickness involvement of the sclera within the inner two thirds
pT3d	Full-thickness invasions into the outer third of the sclera and/or invasion into or around emissary channels
pT4	Evidence of extraocular tumor; tumor at the transected end of the optic nerve, tumor into meningeal spaces around the optic nerve, full-thickness invasion of the sclera with invasion of the episclera, adjacent adipose tissue, extraocular muscle, bone, conjunctiva, or eyelids

Choroidal Invasion
The presence and extent (focal vs massive) of choroidal invasion by tumor should be stated. Differentiation should be made between true choroidal invasion and artifactual invasion due to seeding of fresh tumor cells during post-enucleation retrieval of tumor tissue and/or gross sectioning.

Focal Choroidal Invasion
Defined as a solid nest of tumor that measures less than 3 mm in maximum diameter (width or thickness).

Massive Choroidal Invasion
Defined as a solid tumor nest 3 mm or more in maximum diameter (width or thickness) in contact with the underlying sclera.

TNM Descriptors:

For identification of special cases of TNM or pTNM classifications, the "m" suffix and "y," "r," and "a" prefixes are used. Although they do not affect the stage grouping, they indicate cases needing separate analysis.

pT(m)NM: The "m" suffix indicates the presence of multiple primary tumors in a single site.

ycTNM or ypTNM: 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). It categorizes the extent of tumor actually present at the time of examination; it is not an estimate of tumor prior to multimodality therapy.

rTNM: The "r" prefix indicates a recurrent tumor when staged after a documented disease-free interval.

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

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

- **Specimen Handling:**

  - **Fixation:**

    The minimum fixation time for whole globes with intraocular tumors is 48 hours with a 10:1 ratio of 10% neutral buffered formalin volume to specimen volume. Incisions or windows in the globe are not recommended and are not necessary for adequate fixation. Injection of fixative into the globe is also not recommended.

  - **Sectioning:** *(Figure 2)*

    Following fixation, the optic nerve should be amputated about 2 mm behind the globe if not previously performed in the operating room. The surgical margin should be embedded face down prior to sectioning the globe to ensure that intraocular malignant cells do not contaminate this important surgical margin.
    This margin must be submitted first, prior to additional sectioning.

    The first section of the eye should extend from the pupil through the optic nerve (the "P-O" section). This section is critical for evaluation of the optic nerve for tumor invasion and contains the center of the optic nerve with all the optic nerve structures (optic nerve head, lamina cribrosa, and postlaminar optic nerve). Preferably, this plane will bisect the largest dimension of the tumor, previously identified by transillumination. This section plane should avoid any scleral opening previously made for fresh tumor sampling. The globe is generally sectioned in the horizontal or vertical plane. If the mass cannot be included in these planes, the globe is sectioned obliquely to include tumor, pupil, and optic nerve.

The remaining minor calottes should be additionally sectioned anterior to posterior in a bread-loaf fashion. These segments should be submitted in 1 cassette per calotte on edge to evaluate the choroid for invasion. The P-O section and minor calottes are then embedded in paraffin for a total of 4 cassettes: the optic nerve stump, the P-O section, and the 2 minor calottes.

Note: The macroscopic features of ocular specimens provide valuable information. Gross photography of these valuable specimens is highly recommended and establishes a permanent documentation of the macroscopic features.

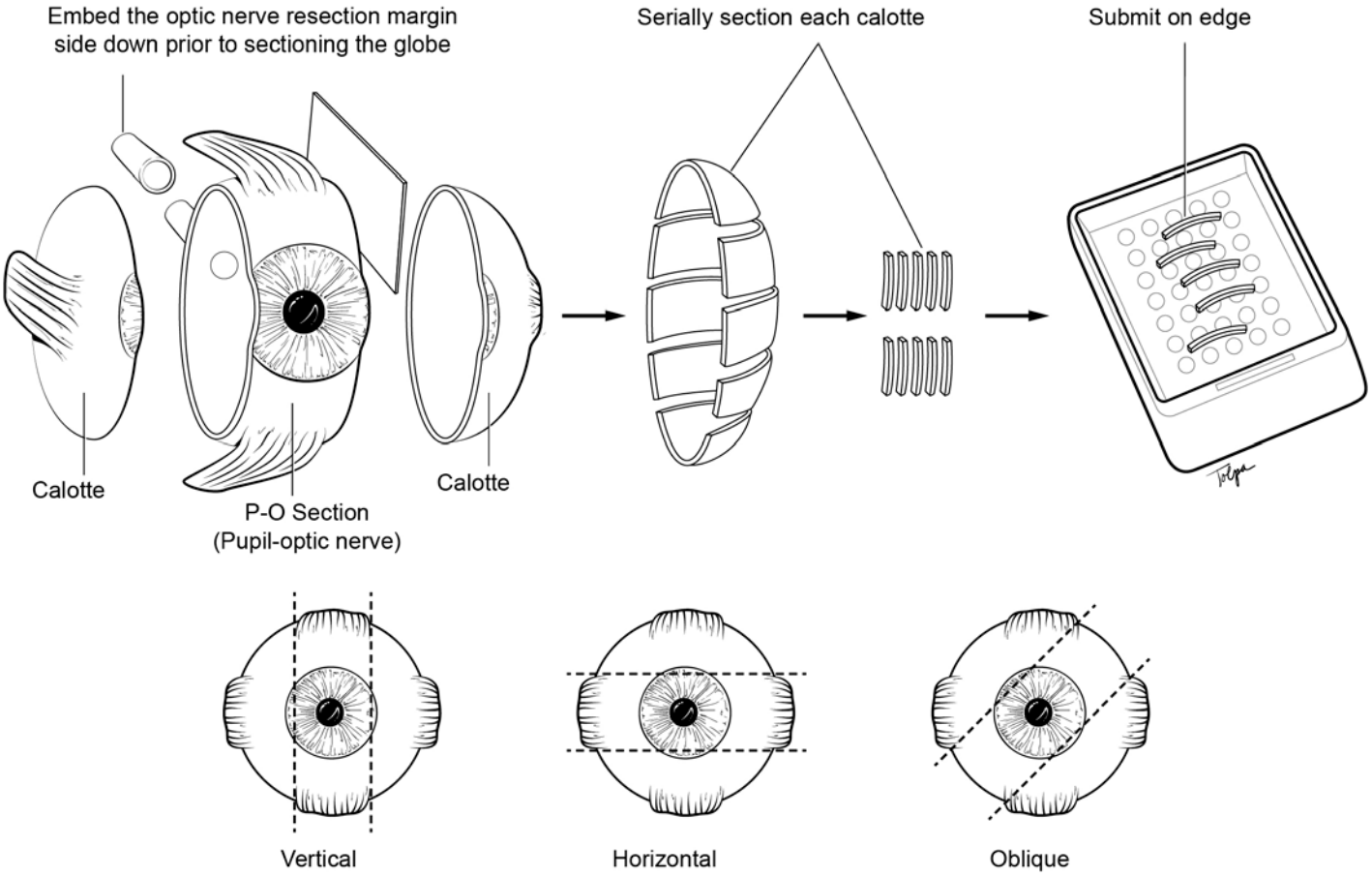

Figure 2: Common Methods of Sectioning the Globe

- **Margins:**

  The most important part of the macroscopic examination of a retinoblastoma enucleation is extremely careful evaluation of the surgical margin at the site of transection of the optic nerve.

  - Specify if tumor is present or not present at the surgical margin of the optic nerve:
    - Apply ink to the surgical margin of the optic nerve.
    - Cut the optic nerve stump off from the sclera with a razor approximately 2 mm behind the globe.
    - Instruct the histotechnologist to embed the nerve stump face down ensuring that sections start at the surgical margin.
  - Specify if there is extrascleral extension of tumor (enucleation specimens).
  - If margins are macroscopically uninvolved by tumor: *(refer to figure 2, page 13)*
    - Optic nerve margin: provide the clearance measurement, declare the specific location(s), and demonstrate for microscopic evaluation, the closest margin approach(s).
      - The surgical margin of the nerve stump should be embedded face down in paraffin for sectioning (i.e., thereby obtaining cross-sections of the nerve, starting at the surgical margin).
    - Extrascleral and choroidal extension (for enucleation specimens): provide the clearance measurement, declare the specific location(s), and demonstrate for microscopic evaluation, the closest margin approach(s).
    - Soft tissue margins (for exenteration specimens): provide the clearance measurement, declare the specific location(s), and demonstrate for microscopic evaluation, the closest margin approach(s).
  - If margins are macroscopically involved by tumor: *(refer to figure 2, page 13)*
    - Optic nerve margin: demonstrate for microscopic examination.
      - The surgical margin of the nerve stump should be embedded face down in paraffin for sectioning (i.e., thereby obtaining cross-sections of the nerve, starting at the surgical margin).
    - Extrascleral and choroidal extension (for enucleation specimens): declare the specific location(s) and demonstrate for microscopic examination.
    - Soft tissue margins (for exenteration specimens): declare the specific location(s) and demonstrate for microscopic examination.

LYMPH NODES ("N" of TNM)

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

Definition of Regional Lymph Node (pN)

pN Category	pN Criteria
pNX	Regional lymph node involvement cannot be assessed
pN0	No lymph node involvement
pN1	Regional lymph node involvement

This category of staging applies only to extraocular extension involving ocular adnexal tissues with lymphatic supply, as there are no known intraocular lymphatics.

- Regional lymph nodes:
  - Preauricular (parotid)
  - Submandibular
  - Cervical

There are no intraocular lymphatics, and lymph node submission is extremely rare. However, if lymph nodes are encountered or submitted, follow the recommendations below:

- Count lymph nodes identified or submitted.
- Specify number of lymph nodes involved.
- Specify site of lymph nodes identified or as submitted.
- Measure lymph nodes in three dimensions or provide a range in size of greatest dimension if multiple.
- Describe the cut surface of the identified lymph nodes.

- **For macroscopically positive lymph nodes**:
  - Give a precise size of the lymph node and tumor implant.
  - Representative sampling of these lymph nodes is adequate.
  - Sample areas where extranodal extension is seen or cannot be excluded.
    - Submit the entire capsule of the lymph node for possible extranodal extension.
  - If possible, submit lymph node sections so that the long axis of the lymph node is demonstrated.

- **For small lymph nodes or macroscopically negative larger lymph nodes**:
  - Submit small lymph nodes in toto.
  - Section larger lymph nodes at 2 mm intervals and entirely submit.
  - If possible, submit lymph node sections so that the long axis of the lymph node is demonstrated.
  - If the lymph node is large but macroscopically negative, submit sequentially so that a size calculation can be established.

- 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
pM1	Distant metastasis with histopathologic confirmation
pM1a	Histopathologic confirmation of tumor at any distant site (e.g., bone marrow, liver, or other)
pM1b	Histopathologic confirmation of tumor in the cerebrospinal fluid or CNS parenchyma

- Retinoblastoma can metastasize through hematogenous routes to various sites, usually the bone marrow and bone.
- Tumor may spread from within the optic nerve or subarachnoid space into the intracranial space. CNS involvement of retinoblastoma portends a worse prognosis than spread to bone marrow.
- Features with prognostic significance for survival include:
  - Invasion of optic nerve
  - Invasion of sclera
  - Invasion of choroid
  - Tumor size
  - Basophilic staining of tumor vessels
  - Seeding of vitreous
  - Degree of differentiation
  - Involvement of anterior segment
  - Growth pattern

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