Disclosures

• None.
1. What happens to my tumor in pathology?

2. How does the pathologist diagnosis my tumor?

3. How does pathology help risk assess my tumor?

4. What happens when my tumor is sent for mutation testing and why is it requested?

5. What information should be in my pathology report?
What happens to my tumor in pathology?
Surgical specimen is received from the OR and logged into computer.

Specimen given to pathologist or pathology assistant to examine.
Tumor is described & measured and margins examined.

Surgeon may request margins to be checked and free of tumor.
Sections of tumor and margins are taken and placed in plastic cassettes so they can be microscopically examined.
The tissue blocks are fixed in formalin and then loaded on a tissue processor overnight.
Tissue processing utilizes graded treatments of formalin, ethanol, xylene and paraffin.
Processed tissue are embedded into a paraffin mold and cooled – resulting in a tissue block.
Paraffin-embedded blocks are loaded and cut using a microtome.
Tissue paraffin ribbons are placed in a warm water bath and then picked up on glass slides.
Unstained slides are stained with Hematoxylin & Eosin (H&E).

Given to pathologist to examine under microscope and generate path report.

May order studies to help confirm diagnosis.
After final diagnosis, both slides and the paraffin blocks from which they are cut are cataloged and stored for future use.
How does the pathologist diagnosis my tumor?
Gastrointestinal Stromal Tumor

- Arise from the interstitial cells of Cajal (ICC)

- ICC have a “pacemaker” function and are important in coordinating peristalsis

CD117 / KIT

Hornick & Lazar. GSI website: Understanding Your Pathology Report for GIST
Sites of Involvement

- Rectum (5%)
- Esophagus (2%)
- Other (colon, mesentery, retroperitoneum)
- Stomach (60%)
- Small intestine (25%)
- Extra-gastrointestinal (<1%): Omentum, mesentery, pelvis and retroperitoneum

Gross Appearance

- Most arise from muscularis propria (outer layer of GI tract)
- Can grow inwards or out or both
- Size varies greatly (median of 10 cm)

Courtesy of Brian Rubin, Cleveland Clinic
Microscopic Features

Spindled Cell

- Tapered edges
- Can have perinuclear vacuoles
Microscopic Features
Epithelioid

- Tumors can also be mixed type
Microscopic Features

Why reported?

- Useful to convey morphology type in case future specimens are encountered
- Sometimes associated with certain clinical and molecular features
Can have unusual histological appearances making diagnosis challenging.
What else can mimic GIST?

- Can also involve GI tract
- Immunohistochemistry can help pathologists to confirm diagnosis

- Leiomyoma
- Leiomyosarcoma
- Schwannoma
- Fibromatosis
- Carcinoma
- Melanoma

Schwannoma
Leiomyosarcoma
What is immunohistochemistry?

- Tumor cells can express different proteins
- Apply different antibodies to unstained slides to detect specific proteins in cells
- If pigment develops (usually brown or red), it is positive for the protein

Positive for CD117

Negative for CD117
# Immunohistochemical Profile of GISTs

<table>
<thead>
<tr>
<th></th>
<th>H&amp;E</th>
<th>CD117 (KIT)</th>
<th>CD34</th>
<th>Smooth muscle actin</th>
<th>S100 protein</th>
<th>Desmin</th>
<th>Pan-keratin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>95%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>70%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>30%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>5%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>&lt;1%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- **CD117 (KIT)+ (95%)**
- **DOG1 + (95%)**
- **CD34 + (70%)**

- **SMA + (30-40%)**
- **Desmin neg**
- **S-100 protein neg**
- **Keratin neg**
CD117(KIT)-negative GIST
- CD117 and DOG1 negative
<table>
<thead>
<tr>
<th>DIAGNOSIS</th>
<th>KIT/DOG1</th>
<th>CD34</th>
<th>Ker</th>
<th>SMA</th>
<th>DES</th>
<th>S-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIST</td>
<td>+</td>
<td>+ (70%)</td>
<td>-</td>
<td>+ (40%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+ (sar)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Melanoma</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Leiomyoma</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Leiomyosarcoma</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>Schwannoma</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fibromatosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
</tr>
</tbody>
</table>
How does pathology help risk assess my tumor?
Risk Assessment

- How likely will my tumor metastasize?
- Behavior of GIST are difficult to predict
- Have tendency for intra-abdominal spread and metastasis to liver. Rarely to lymph nodes.
- Although many soft tissue tumors are graded (aka. tumor aggressiveness/metastatic potential) under FNCLCC system, this does not apply to GIST
GIST
Risk Assessment

1. Size:
What is the largest measurement size (cm)?

2. Anatomic Site:
Where is it occurring?
3. Mitotic Activity: How many cells are dividing in 5 mm² area?

<table>
<thead>
<tr>
<th>Tumor Parameters</th>
<th>Risk of Progressive Disease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mitotic Index</strong></td>
<td><strong>Size</strong></td>
</tr>
<tr>
<td>≤ 5 per 50 hpf</td>
<td>≤ 2 cm</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 ≤ 5 cm</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 ≤ 10 cm</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm</td>
</tr>
<tr>
<td>&gt; 5 per 50 hpf</td>
<td>≤ 2 cm</td>
</tr>
<tr>
<td></td>
<td>&gt; 2 ≤ 5 cm</td>
</tr>
<tr>
<td></td>
<td>&gt; 5 ≤ 10 cm</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm</td>
</tr>
</tbody>
</table>

Helps determine a probability of behavior for primary tumors

***Modified from Miettinen & Lasota, *Semin Diagn Pathol*, 2006 by Dr. Chris Corless, OHSU. Data based on long-term follow-up of 1055 gastric, 629 small intestinal, 144 duodenal and 111 rectal GISTs.[Miettinen et al. 2005 and 2006]***
Overall Survival by Risk Group

- Normal pop.
- Very low
- Low
- Intermediate
- High
- Overtly malignant

Estimated proportion surviving vs. Years since diagnosis.
What happens when my tumor is sent for mutation testing and why is it requested?
**KIT** (4p12) / **PDGFRA** (7p22.3)

- Majority of GISTs are characterized with recurrent mutations involving either gene
- Both encode for proteins which are located on the cell surface
- Plays a role in cell growth and survival
• Most often involves *KIT*
• Certain portion of the genes (i.e. exons) encode for different parts of the protein are characteristically mutated
• Non-*KIT/PDGFRA* mutated = Wild type (WT) and may have alternative gene mutations (ex. *BRAF V600E*)
• May affect response to therapy
Mutation Types

• Many types of mutations: point mutations, deletions, duplications, etc.
• Reported with area of protein affected (ex. V559_V560del, A502_Y503dup, V560D)
**KIT/PDGFRA Testing**

*Formalin Fixed Paraffin Embedded (FFPE)*

- **Diagnosis confirmed**
- **H&E X1**
- **Review and mark slide for microdissection**
- **Unstained Slides**
  - Overlay on H&E and scrape tissue from unstained
  - Extract DNA for **KIT** and **PDGFRA** testing

[Source: https://ghr.nlm.nih.gov/primer/basics/dna]
Exon 11
V559_V560del

Sanger Sequencing
NM_000222.2(KIT):c.1725_1739del p.Q575_D579del

a 15-bp inframe deletion in exon 11 of KIT causing a loss of 5 amino acids.
Role of Mutation Testing

**Pathologist**
Confirm diagnosis or help in challenging cases:
- KIT IHC negative
- Unusual histological features

**Medical Oncologist**
Predict tumor behavior to therapy:
- Sensitive
- Require different dosage
- High risk of failing standard therapy
Confirmation of Diagnosis in KIT IHC (-) Tumors
Predicting who may need higher doses of therapy

Progression free survival
Patients harboring KIT exon 9 mutations
Require higher doses of imatinib

Recommendations for adjuvant imatinib therapy by integration of the risk assessment (based on modified NIH classification) and genotype


*Metastatic/locally advanced GIST with KIT ex. 9 mutations respond better to 800 mg imatinib daily (compared with the standard 400 mg). Therefore, increased dose may be considered in the adjuvant setting.
Treatment effect

Pre-Treatment

Post Treatment

Decrease cellularity
Myxohyaline stroma
• Different mutations can exist within the same nodule and multiple tumor nodules
• Can acquire additional mutations
• Can also acquire skeletal muscle differentiation or de-differentiate
Familial GIST

• Rare germline mutation in *KIT* or *PDGFRA*, autosomal dominant

• Multiple tumors and early with hyperplasia of cajal cells

• May have skin lesions or swallowing difficulty
GIST associated with Neurofibromatosis

- Similar morphology but generally wild type for *KIT/PDGFRA*
- Different molecular mechanism for these tumors
- Indolent than conventional types
GISTs with Multinodular/Plexiform Growth Patterns

- Epithelioid or mixed morphology
- Pediatric (<20 yo) but sometimes in adults
- Distant metastases common including lymph nodes
- Clinically indolent
- Wild-type for *KIT/PDGFRA*

Courtesy of Jason Hornick, BWH/Harvard, Boston, MA
• Pediatric GISTs
  – Female predominance (peak 2\textsuperscript{nd} decade)
  – Indolent, but late metastases common
  – Molecular genetic basis unknown

• Carney Triad
  – Gastric GIST, pulmonary chondroma, paraganglioma
  – Molecular genetic basis unknown

• Carney-Stratakis Syndrome
  – Gastric GIST and paraganglioma
  – Germline mutations in succinate dehydrogenase subunit genes (\textit{SDHA, SDHB, SDHC, or SDHD})
What information should be in my pathology report?
Protocol for the Examination of Resection Specimens From Patients With Gastrointestinal Stromal Tumor (GIST)

Version: GIST Resection 4.1.0.0  Protocol Posting Date: August 2019

CAP Laboratory Accreditation Program Protocol Required Use Date: May 2020

Includes pTNM requirements from the 8th Edition, AJCC Staging Manual

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></td>
</tr>
<tr>
<td>Tumor Type</td>
<td>Description</td>
</tr>
<tr>
<td>Gastrointestinal stromal tumor</td>
<td></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</td>
</tr>
<tr>
<td>Local excision</td>
</tr>
<tr>
<td>Primary resection specimen with no residual tumor (eg, following neoadjuvant therapy)</td>
</tr>
<tr>
<td>Cytologic specimens</td>
</tr>
</tbody>
</table>

Authors
Javier A. Laurini, MD*; Charles D. Blanke, MD; Kumarasen Cooper, MBChB, DPhil, FRCPath; George D. Demetri, MD; Ronald P. Dematteo, MD; Christopher D.M. Fletcher, MD, FRCPath; John R. Goldblum, MD; Thomas Krausz, MD, FRCPath; Jerzy Lasota, MD, PhD; Alexander Lazar, MD, PhD; Robert G. Maki, MD, PhD; Markku Miettinen, MD, PhD; Amy Noffsinger, MD; Jordan E. Olson, MD; Brian P. Rubin, MD, PhD; Mary K. Washington, MD, PhD

With guidance from the CAP Cancer and CAP Pathology Electronic Reporting Committees.

* Denotes primary author. All other contributing authors are listed alphabetically.
What was the procedure?

GASTROINTESTINAL STROMAL TUMOR (GIST): Resection

Procedure
___ Local excision
___ Resection
   Specify type (eg, partial gastrectomy): ____________________________
___ Metastasectomy
___ Other (specify): ____________________________
___ Not specified

Tumor Site (Note A)
Specify (if known): ____________________________
___ Not specified

Tumor Size
Greatest dimension (centimeters): ___ cm
+ Additional dimensions (centimeters): ___ x ___ cm
___ Cannot be determined (explain): ____________________________

Tumor Focality
___ Unifocal
___ Multifocal
   Specify number of tumors: ______
   Specify size of tumors: ____________________________

Where was it located?

How big was it?

How many tumors seen?
<table>
<thead>
<tr>
<th>Histologic Type</th>
<th>What is the diagnosis and what did it look like?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal stromal tumor, spindle cell type</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal stromal tumor, epithelioid type</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal stromal tumor, mixed</td>
<td></td>
</tr>
<tr>
<td>Gastrointestinal stromal tumor, other (specify):</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mitotic Rate</th>
<th>How many mitotic figures were seen?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specify: ___ /5 mm²</td>
<td></td>
</tr>
<tr>
<td>___ Cannot be determined (explain):</td>
<td></td>
</tr>
</tbody>
</table>

Note: The required total count of mitoses is per 5 mm² on the glass slide section. With HPF is equivalent to 5 mm². Most modern microscopes with wider 40X lenses/fields require encompass 5 mm². If necessary, please measure field of view to accurately determine actual number of fields required to be counted on individual microscopes to encompass 5 mm².

<table>
<thead>
<tr>
<th>Necrosis</th>
<th>How much of tumor was dead?</th>
</tr>
</thead>
<tbody>
<tr>
<td>___ Not identified</td>
<td></td>
</tr>
<tr>
<td>___ Present &amp; Extent: ___%</td>
<td></td>
</tr>
<tr>
<td>___ Cannot be determined</td>
<td></td>
</tr>
</tbody>
</table>
Tumor surgically cleared?

Was there any lymph node involvement (rare)?

Grade and Risk assessment

Histologic Grade (Note B)
- G1: Low grade; mitotic rate ≤5/5 mm²
- G2: High grade; mitotic rate >5/5 mm²
- GX: Grade cannot be assessed

Risk Assessment (Note C)
- None
- Very low risk
- Low risk
- Moderate risk
- High risk
- Overtly malignant/metastatic
- Cannot be determined

Margins
- Cannot be assessed
- Uninvolved by GIST
  Distance of tumor from closest margin (millimeters or centimeters): ___ mm or ___ cm
  Specify margin (if known): ____________________________
- Involved by GIST
  Specify margin(s) (if known): ____________________________

Regional Lymph Nodes (Note D)
- No lymph nodes submitted or found

Lymph Node Examination (required only if lymph nodes are present in specimen)

Number of Lymph Nodes Involved: _____
- Number cannot be determined (explain): ____________________________

Number of Lymph Nodes Examined: _____
- Number cannot be determined (explain): ____________________________
Tumor Stage

- **Standard measure of tumor burden** – How much tumor?
- **Pediatric, familial and syndromic GIST are not staged.**

---

**Pathologic Stage Classification (pTNM, AJCC 8th Edition) (Note E)**

*Note: Reporting of pT, pN, and (when applicable) pM categories is based on information available to the pathologist at the time the report is issued. Only the applicable T, N, or M category is required for reporting; their definitions need not be included in the report. The categories (with modifiers when applicable) can be listed on 1 line or more than 1 line.*

**TNM Descriptors (required only if applicable) (select all that apply)**

- ___ m (multiple)
- ___ r (recurrent)
- ___ y (posttreatment)

**Primary Tumor (pT)**

- ___ pTX: Primary tumor cannot be assessed
- ___ pT0: No evidence of primary tumor
- ___ pT1: Tumor 2 cm or less
- ___ pT2: Tumor more than 2 cm but not more than 5 cm
- ___ pT3: Tumor more than 5 cm but not more than 10 cm
- ___ pT4: Tumor more than 10 cm in greatest dimension

**Regional Lymph Nodes (pN) (Note D) (required only if lymph nodes submitted in this case)**

- ___ pN0: No regional lymph node metastasis
- ___ pN1: Regional lymph node metastasis

*When no lymph nodes are present (as is often the case with resection for GIST), the pathologic ‘N’ category is not assigned (pNX is not used for GIST) and should not be reported.*

**Distant Metastasis (pM) (Note D) (required only if confirmed pathologically in this case)**

- ___ pM1: Distant metastasis
  - Specify site(s), if known: ______________________

+ Additional Pathologic Findings
+ Specify: ______________________
Ancillary Studies (Note F)

Note: The CAP GIST Biomarker Template can be used for reporting biomarkers requested for this resection specimen. Pending biomarker studies should be listed in the Comments section of this report.

+ Immunohistochemical Studies (select all that apply)
  ___ Not performed
  + ___ KIT (CD117)
    + ___ Positive
    + ___ Negative
  + ___ DOG1 (ANO1)
    + ___ Positive
    + ___ Negative
  + ___ SDHB
    + ___ Intact
    + ___ Deficient
  + ___ SDHA
    + ___ Intact
    + ___ Deficient

___ Pending
___ Other (specify): ________________

+ Molecular Genetic Studies (eg, KIT, PDGFRA, BRAF, SDHA/B/C/D, or NF1 mutational analysis)
+ ___ Submitted for analysis; results pending
+ ___ Performed, see separate report: ________________
+ ___ Performed
  + Specify method(s) and results: ________________
+ ___ Not performed
Preresection Treatment (select all that apply)
+___ No known preresection therapy
+___ Previous biopsy or surgery (specify): ____________________________
+___ Systemic therapy performed (specify type): ________________________
+___ Therapy performed, type not specified
+___ Not specified

Treatment Effect (Note G)
___ No known presurgical therapy
___ Not identified
___ Present
   + Specify percentage of viable tumor: ___%
___ Cannot be determined

+ Comment(s)
Future Direction

- Genomic instability and complexity can provide prognostic information

- **CINSARC** (Complexity Index in Sarcomas): Prognostic gene expression signature using multiple genes related to mitoses and chromosome management
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