GIST 201: Understanding Your Pathology Report with \textit{KIT} / \textit{PDGFRA} Genotyping

GSI Patient Summit – Saturday 14 September 2013

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Section of Soft Tissue/Sarcoma Pathology

Faculty, Sarcoma Research Center
GIST Pathology: Lecture Overview

1. What happens to my tumor in pathology?
2. What information is in my pathology report?
3. Why is this information there?
4. What is the evidence that the information is useful?
5. What is mutational testing?
What happens to my tumor in pathology?
Tumor sample is received from the OR and logged into computer.

Tumor is examined by a pathologist.
GIST - Gross Appearance

Courtesy of Brian Rubin, Cleveland Clinic
Tumor is sampled and placed in plastic cassettes for further processing.

Tumor is also given to cytogenetics, tumor bank, molecular diagnosis and electron microscopy when appropriate.
The tissue blocks are fixed in formalin and then loaded on a tissue processor overnight.
Tissue processing is done overnight and utilizes graded treatments of formalin, ethanol, xylene and paraffin.
Blocks are retrieved from the tissue processor.
The tissue fragments are embedded in a paraffin mold and cooled – the result being a tissue block.
The paraffin-embedded blocks are loaded and cut using a microtome.
Tissue paraffin ribbons are placed in a warm waterbath and the picked up on glass slides.
The unstained slides can be used for H&E, special stains, immuno-histochemistry, molecular studies, etc.
Most slides are H&E (hematoxlin & eosin) stained, given coverslips, organized and delivered to the proper pathologist.
Additional unstained slides can be cut at a later time.
After final diagnosis, both slides and the paraffin blocks from which they are cut are cataloged and stored for future use.
What information is in my pathology report?
Protocol for the Examination of Specimens From Patients With Gastrointestinal Stromal Tumor (GIST)

Based on AJCC/UICC TNM, 7th edition
Protocol web posting date: June 2012

Procedures
- Biopsy
- Resection

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For the Members of the Cancer Committee, College of American Pathologists

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Surgical Pathology Cancer Case Summary

Protocol web posting date: June 2012

GASTROINTESTINAL STROMAL TUMOR (GIST): Resection

Select a single response unless otherwise indicated.

Procedure
___ Excisional biopsy
___ Resection
    Specify type (e.g., partial gastrectomy):
___ Metastasectomy
___ Other (specify):
___ Not specified

Tumor Site
Specify (if known):
___ Not specified

Tumor Size
Greatest dimension: ___ cm
+ Additional dimensions: ___ x ___ cm
___ Cannot be determined (see “Comment”)

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

GIST Subtype
___ Spindle cell
___ Epithelioid
___ Mixed
___ Other (specify):

—
Mitotic Rate
Specify: ___ /50 HPF

Note: The required total count of mitoses is per 5 mm² on the glass slide section. With the use of older model microscopes, 50 HPF is equivalent to 5 mm². Most modern microscopes with wider 40X lenses/fields require only 20 HPF to embrace 5 mm². If necessary please measure field of view to accurately determine actual number of fields required to be counted on individual microscopes to count 5 mm².

+ Necrosis
  + ___ Not identified
  + ___ Present
    + Extent: ___%
  + ___ Cannot be determined
Histologic Grade (Note B)
- **G0**: Grade cannot be assessed
- **G1**: Low grade; mitotic rate ≤5/50 HPF
- **G2**: High grade; mitotic rate >5/50 HPF

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

Margins
- Cannot be assessed
- Negative for GIST
  - Distance of tumor from closest margin: ___ mm or ___ cm
- Margin(s) positive for GIST
  - Specify margin(s): ________________

Pathologic Staging (pTNM) (Note G)

TNM Descriptors (required only if applicable) (select all that apply)
- **m** (multiple)
- **r** (recurrent)
- **y** (posttreatment)

Primary Tumor (pT)
- **pT0**: Primary tumor cannot be assessed
- **pT0**: No evidence for 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)
- **N**ot applicable
- **pN0**: No regional lymph node metastasis
- **pN1**: Regional lymph node metastasis

Distant Metastasis (pM) (Note D)
- **N**ot applicable
- **pM1**: Distant metastasis
  - Specify site(s), if known: ________________

* Additional Pathologic Findings
  * Specify: ________________
Ancillary Studies (select all that apply) (Note E)

Immunohistochemical Studies
___ KIT (CD117)
    ___ Positive
    ___ Negative
___ Others (specify): ________________________
___ Not performed

Molecular Genetic Studies (e.g., KIT or PDGFRA 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 therapy
___ Previous biopsy or surgery
    Specify: ________________________
___ Systemic therapy performed
    Specify type: ________________________
___ Therapy performed, type not specified
___ Unknown

+ Treatment Effect (Note F)
+ Specify percentage of viable tumor: ___%
The many faces of GIST
**Immunohistochemical Profile of GIST**

<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>70%</td>
<td>30%</td>
<td>5%</td>
<td>2%</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

- **KIT (CD117) +ve (95%)**
- **CD34 +ve (70%)**
- **SMA +ve (30-40%)**
- **Desmin −ve**
- **S-100 protein −ve**
- **Keratin −ve**

KIT immunoreactivity in GIST
KIT-negative GIST
Risk assessment in GIST
GIST – Prognostic Factors

Size
Mitotic Rate
Anatomic Location
Pleomorphism
Cellularity
Necrosis
Mucosal Invasion
Proliferation Markers (Ki-67, Mib-1, PCNA, etc)
DNA Flow Cytometry
Image Analysis
Nuclear Organizer Regions

Problem – Small GISTs without mitoses can metastasize!
### NIH Consensus Risk Assessment

<table>
<thead>
<tr>
<th>Risk Level</th>
<th>Size</th>
<th>Mitotic Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very Low Risk</td>
<td>&lt; 2 cm</td>
<td>&lt; 5/50 HPF</td>
</tr>
<tr>
<td>Low Risk</td>
<td>2-5 cm</td>
<td>&lt; 5/50 HPF</td>
</tr>
<tr>
<td>Intermediate Risk</td>
<td>&lt; 5 cm</td>
<td>6-10/50 HPF</td>
</tr>
<tr>
<td></td>
<td>5-10 cm</td>
<td>&lt; 5/50 HPF</td>
</tr>
<tr>
<td>High Risk</td>
<td>&gt; 5 cm</td>
<td>&gt; 5/50 HPF</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm</td>
<td>Any Mitotic Rate</td>
</tr>
<tr>
<td></td>
<td>Any Size</td>
<td>&gt; 10/50 HPF</td>
</tr>
</tbody>
</table>

Fletcher et al., Hum Pathol, 2002
GIST: Sites of Involvement

- Rectum (5%)
- Esophagus (2%)
  Other (colon, mesentery, retroperitoneum)
- Stomach (60%)
- Small intestine (25%)
- Other (colon, mesentery, retroperitoneum) (<1%)

Omentum, mesentery, pelvis and retroperitoneum = EGIST (<1%)

Hornick & Lazar. GSI website: Understanding Your Pathology Report for GIST.
**2007/2010 NCCN GIST Risk Assessment Guidelines***

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Parameters</th>
<th>Risk of</th>
<th>Progressive</th>
<th>Disease# (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Size</td>
<td>Gastric</td>
<td>Duodenum</td>
</tr>
<tr>
<td>Mitotic</td>
<td>≤ 2 cm</td>
<td>None (0%)</td>
<td>None (0%)</td>
<td>None (0%)</td>
</tr>
<tr>
<td>Index</td>
<td>&gt; 2 ≤ 5 cm</td>
<td>Very low (1.9%)</td>
<td>Low (8.3%)</td>
<td>Low (4.3%)</td>
</tr>
<tr>
<td>≤ 5 per 50 hpf</td>
<td>&gt; 5 ≤ 10 cm</td>
<td>Low (3.6%)</td>
<td>(Insuff. data)</td>
<td>Moderate (24%)</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 cm</td>
<td>Moderate (10%)</td>
<td>High (34%)</td>
<td>High (52%)</td>
</tr>
<tr>
<td>Mitotic</td>
<td>≤ 2 cm</td>
<td>None*</td>
<td>(Insuff. data)</td>
<td>High*</td>
</tr>
<tr>
<td>Index</td>
<td>&gt; 2 ≤ 5 cm</td>
<td>Moderate (16%)</td>
<td>High (50%)</td>
<td>High (73%)</td>
</tr>
<tr>
<td>&gt; 5 per 50 hpf</td>
<td>&gt; 5 ≤ 10 cm</td>
<td>High (55%)</td>
<td>(Insuff. data)</td>
<td>High (85%)</td>
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<tr>
<td></td>
<td>&gt; 10 cm</td>
<td>High (86%)</td>
<td>High (86%)</td>
<td>High (90%)</td>
</tr>
</tbody>
</table>

***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 GIST

Miettinen *et al.* 2005 and 2006
## 2007/2010 NCCN GIST Risk Assessment Guidelines***

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<th>Tumor Parameters</th>
<th>Risk of Progressive Disease# (%)</th>
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<tr>
<td></td>
<td>Gastric</td>
<td>Duodenum</td>
<td>Jejunum/Ileum</td>
</tr>
<tr>
<td>Mitotic</td>
<td>≤ 2 cm</td>
<td>None (0%)</td>
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Miettinen *et al.* 2005 and 2006
GIST - Recurrence-Free Survival Following Surgical Treatment of Primary GIST

- Recurrence-free survival is predicted by tumor size and mitotic index

FNCLCC Grading

• All three numbers are summated to determine degree of differentiation

Grade 1: 2-3
Grade 2: 4-5
Grade 3: 6-8

• Proven to correlated well with survival

• **Mitotic Count.** In the most mitotically active area, ten successive high-power fields (at 400x magnification=0.1734 mm$^2$) using a 40x objective.

1. 0-9 mitoses per 10 HPFs
2. 10-19 mitoses per 10 HPFs
3. >20 mitoses per 10 HPFs

• **Tumor necrosis.** Evaluated on gross examination and validated with histological sections

0. No tumor necrosis
1. <50% tumor necrosis
2. >50% tumor necrosis

• **Degree of Differentiation.** 1-3
GIST - Overall Survival by Risk Group

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

Years since diagnosis

Estimated proportion surviving
Treatment can cause big changes.
Treatment effect

Pre-Imatinib

Post-Imatinib (8 weeks therapy)
Long term Imatinib Tx
Long term Imatinib Tx
What do we mean by Genotyping or Mutational Testing?
What Are Genes?

- Tissues: specialized structures made up of cells
- Cells: building units, made up of cytoplasm and nucleus
- Nucleus: instructions/blueprint for cells
- Genes: carry the hereditary characteristic of cells
- Chromosome: made up of DNA and other proteins
- DNA: molecule encodes genetic data
Human Genetics

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23 chromosomes

---

23 chromosomes

---

46 chromosomes
Human Genetics
Chromosome Structure And Expression

**Genes:** are made up of sequences of exons and introns

- **Introns:** DNA sequence of a gene that encodes for protein
- **Exons:** parts of genes that do not encode for protein

**Gene expression:**
1. Transcription: formation of mRNA
2. Translation: formation of protein
Cellular Differentiation
Same Genes
Different Cells

- Somatic cells share similar genetic composition
- Some genes are expressed, others are not
- Gene expression determines shape and function of cells
Human Genome Project

- All DNA sequence in the 23 pairs of chromosomes
- <2% of genome encode for proteins
- >98% of genome do not encode for proteins
Regulation Of Gene Expression

- Level of regulation:
  - Transcription of genes
  - Post transcription of genes
  - Translation of mRNA
  - Protein degradation
Regulation Of Proliferation

• Cell proliferation restricted under normal circumstances. Cells enter cell cycle to proliferate.
• Apoptosis to remove excess and damaged cells.
• Genes regulate cell cycle and apoptosis.
Mutation

• Mutation: change in genome structure of a cell that may or may not alter its phenotypic properties.

• Consequences:
  – None
  – Loss of function
  – Gain of function

• Causes:
  – Radiation
  – Chemicals
  – Viruses
  – Genetic aberrations
Genotyping In GIST

- Genotyping: studying genetic constitution by determining differences in the genetic make-up of an individual and comparing it to a reference sequence.
- Genotyping work up for GIST cases:
  - *KIT* (muts in 80-85%)
  - *PDGFRA* (muts in 10-15%)
  - Wild type
**KIT** Gene Mutation In GIST

- KIT (also known as CD117) is a cell surface protein.
- It plays a role in cell survival, proliferation and differentiation.
- It is found to be mutant in 80-85% of GIST tumors.
- Mutation leads to gain of function.
PDGRA Mutation In GIST

• Platelet derived growth factor receptor that binds GFRA and promote proliferation of blood vessel cells and other mesenchymal cells.

• The gene is mutated in 10-15% of GIST tumors.
What is KIT?

- Type III receptor tyrosine kinase
- Chromosome 4q
- Proliferation & maintenance
  - germ cells
  - hematopoietic (mast) cells
  - melanocytes
  - interstitial cells of Cajal.
Normal KIT Function

Ligand Dependent Activation
GISTs Possess Ligand Independent Activating Mutations in *KIT* Exon 11

All mutations - whether involving base pair substitutions, deletions or duplications - preserve the open reading frame.

Hirota et al., Science 1998
**KIT** mutations are activating and oncogenic!

Constructs harboring **KIT** mutants are constitutively phosphorylated and kinase is constitutively activated in Ba/F3 cells.

Ba/F3 cells harboring **KIT** mutant constructs grow autonomously in culture (normally growth factor dependent) and cause tumors in nude mice.
A minority of GISTs possess mutations in *KIT* exons 9, 13, & 17
Activating $KIT$ Mutations in GISTs

Ligand Independent Activation
Imatinib Mesylate

Formula: $\text{C}_{30}\text{H}_{35}\text{N}_7\text{SO}_4$

MW: 589.7

- Rational drug design
  - 2-phenylamino pyrimidine
  - Based on structure of ATP binding site
  - Highly water soluble
  - Oral bioavailability

Inhibitor of selective tyrosine kinases
- bcr-abl
- PDGF-R
- c-kit

Potent ($\text{IC}_{50} \approx 0.1\mu\text{M}$)
KIT Signaling – WT Cells

Proliferation
Survival
Adhesion
Invasion
Metastasis
Angiogenesis
Oncogenic KIT Signaling

GI Stromal Tumors

Proliferation
Survival
Adhesion
Invasion
Metastasis
Angiogenesis

ATP

SIGNALING

courtesy of C. Corless & M. Heinrich
Imatinib Inhibits KIT Signaling

courtesy of C. Corless & M. Heinrich
Exon 11
V559_V560del
Exon 9
A502_Y503dup
Detection of SNV in KIT Exon 10, currently not covered by Sanger

Confirmation by Sanger
ATG→CTG, M541L
KIT EXON 10

75% Tumor

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Position</th>
<th>Gene Symb</th>
<th>Ploidy</th>
<th>Ref</th>
<th>Variant</th>
<th>VarFreq</th>
<th>Coverage</th>
<th>RefCov</th>
<th>VarCov</th>
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<tbody>
<tr>
<td>chr4</td>
<td>5559246</td>
<td>KIT</td>
<td>Het</td>
<td>A</td>
<td>C</td>
<td>63.42</td>
<td>1077</td>
<td>389</td>
<td>683</td>
</tr>
</tbody>
</table>
Thank You

- Brian Rubin, Cleveland Clinic
- Jason Hornick, Brigham & Women’s Hospital/Harvard
- Michael Heinrich & Chris Corless, University of Oregon
- Jon Trent, University of Miami
- Ghadah Al-Saanna, Sarcoma Path Visiting Faculty
- Many colleagues at UTMDACC