GIST Summit 2010

Diagnosis, Prognosis and KIT/PDGFRα Genotyping in Gastrointestinal Stromal Tumors

Saturday Sept 25 2010
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Sections of Sarcoma Pathology & Dermatopathology
Gastrointestinal Stromal Tumors

Role of Pathologic Diagnosis and Risk Assessment

Mutation Analysis
Gastrointestinal Stromal Tumor

- Arise from the interstitial cells of Cajal (ICC)
- ICC are important in coordinating peristalsis
GIST

Sites of Involvement

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

Gross Appearance

- Most originate from muscularis propria (muscle layers)
- Size varies greatly (median of 10 cm)
- Can grow inwards or out

Courtesy of Brian Rubin
GIST
Morphology

- Spindle cell
- Epithelioid
- Mixed
Mimics of GIST

Carcinoma
Melanoma
Leiomyoma
Leiomyosarcoma
Schwannoma
Fibromatosis
### Immunohistochemical Profile of GISTs

<table>
<thead>
<tr>
<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>95%</td>
<td>70%</td>
<td>30%</td>
<td>5%</td>
<td>2%</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

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

**DOG1 +**
- (95% / 40% of KIT neg GIST)

Courtesy of Brian Rubin
GISTs

Clinical Behavior

Behavior is difficult to predict.

Most aggressive GISTs metastasize within 5 yrs.

Small subset may metastasize up to 20 yrs after presentation.

Tendency for intra-abdominal spread and metastasis to liver.

*Never metastasize to lymph nodes.
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
## 2007 NCCN GIST

### Risk Assessment Guidelines

<table>
<thead>
<tr>
<th>Tumor Parameters</th>
<th>Risk of Progressive Disease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size</td>
</tr>
<tr>
<td>Mitotic Index ≤ 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>
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<tr>
<td></td>
<td>&gt; 10 cm</td>
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***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

Risk Groups

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

Years since diagnosis

Estimated proportion surviving
GIST Reporting

- Size
- Site of Involvement
- Mitotic Count (per 50 hpfs)
- Resection margins
- Document metastases
KIT/PDGFRA Genotyping
Majority (86%) of GISTs are characterized with recurrent mutations involving the gene *KIT* or *PDGFRA*

- Both genes encode for proteins which are located on the cell surface
- Plays a role in cell growth and survival
- Regulated by a cytokine, Stem Cell Factor (SCF)
Normal activation by stem cell factor

Abnormal self-activation resulting from mutation

\[ \text{SCF} \quad \text{SCF} \quad \text{SCF} \quad \text{SCF} \]

\[ \text{Regulated signaling cascade} \quad \text{Unregulated, constitutive signaling cascade} \]

\[ \text{Kit} \quad \text{Kit} \quad \text{Kit} \quad \text{Kit} \]
• Most have mutations in KIT
• Certain portion of the genes (i.e. exons) encode for different parts of the protein are characteristically mutated in GISTs
• Beneficial to know which exons are effected

N=950 GISTs Tumors Analyzed In Heinrich & Corless Labs

KIT (78.5%)
- Exon 9
- Exon 11
- Exon 13
- Exon 17

PDGFRA (7.5% total)
(35% of KIT-WT)
- Exon 12
- Exon 14
- Exon 18
Mutation Types

- Many types of mutations
- Point mutations, deletions, duplications, etc.
- Reported with area of protein affected (ex. V559_V560del, A502_Y503dup, V560D)
- Can also be important to know specific areas of protein involved within an exon
- Area of on-going research

N=58 Exon 11 (UTMDACC)
Analysis of *KIT/PDGFRα* Genotyping

Formalin Fixed Paraffin Embedded (FFPE)

- Immunohistochemistry (CD117, CD34, SMA, Des, S100, Pan-K)
- H&E X1
- Review and mark slide for microdissection
- Unstained x 10

Overlay on H&E and scrape tissue from unstained

Extract DNA for *KIT* testing (11, 9, 13, 17)
(theoretical 1 in 5 cells – 20%)
Clinical Use of Kinase Genotyping of GISTs

- Genotyping of GISTs for *KIT* and *PDGFRA* mutations may be useful for:
  1. Confirmation of diagnosis KIT IHC (-) GISTs
  2. Prediction of clinical response to imatinib and those that may require different dosage of imatinib
    - ex. exon 9
  3. Triage patients who are at high risk of failing imatinib therapy to enroll in other clinical trials
    - ex. PDGFRA D842V mutations and Wildtype
Confirmation of Diagnosis in KIT IHC (-) Tumors

**Progression free survival**

Patients harboring KIT exon 9 mutations

Require higher doses of imatinib

<table>
<thead>
<tr>
<th>O</th>
<th>N</th>
<th>Number of patients at risk:</th>
<th>Treatmen</th>
</tr>
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<tbody>
<tr>
<td>26</td>
<td>27</td>
<td>14  10  9  6  4  3  1  0</td>
<td>400 mg</td>
</tr>
<tr>
<td>21</td>
<td>31</td>
<td>26  21  20  18  14  9  8  6</td>
<td>800 mg</td>
</tr>
</tbody>
</table>
Frequency and Clinical Significance of *KIT*

**KIT exon 11:** (60–70%) Arise anywhere in GI tract. Most responsive to Imatinib.

**KIT exon 9:** (5–15%) Small intestine. Respond to Imatininib at higher doses.

**KIT exon 13:** (1%) Clinical responses to IM observed but uncharacterized. (**IM resistance point mutations)**

**KIT exon 17:** (1%) Clinical responses to IM observed but uncharacterized. (**IM resistance point mutations**)
Frequency and Clinical Significance of PDGFRA and being WT

**PDGFRA exon 12:** (1%) Rarely originate from the intestine. Clinical responses to IM observed.

**PDGFRA exon 14:** (<1%) Unknown, only few tumors described in the literature.

**PDGFRA exon 18:** (5%) Most originate from the stomach. D842V Resistant to IM.

**Wild type:** (10–15%) Primary resistance to imatinib more common; 40% respond to IM.
Role of KIT genotyping and Resistance

- **Primary Resistance**: 10-15% will not respond as well to imatinib – intrinsically resistant
  - ex. Wildtype (indicate other mechanisms involved), PDGFRA D842V
- **Secondary Resistance**: 50-70% of patients on imatinib will progress and develop resistance
  - Most common cause is the development of a second mutation
  - Most often involve exons 13 and 17 effecting the activation A loop or ATP binding domain resulting in shift to active confirmation or blocking imatinib binding
5 days Imatinib
Long term Imatinib Treatment
Secondary Resistance

- In advancing disease, multiple clones can exist within the same tumor nodule and within separate tumor nodules; each with different mutations
- Very heterogeneous
- Mutation testing does not typically need to be done for secondary resistance
Other Mechanisms of Resistance

• KIT Amplification
• BRAF mutations (KIT Wildtype)
• Insulin Growth Factor
• Loss of Heterozygosity
• AXL overexpression
• Heat Shock Protein 90
• Decreased absorption of imatinib
Familial GIST

- Germline mutation in exon 11.

Familial GIST

Gross Pathology

GISTs associated with Neurofibromatosis

- Similar morphology.
- Mutations are different.
  - Wild type for $KIT$
- Different mechanism for these tumors.
  - IGFR inhibitors?
Thank You.

- Dr. Alexander Lazar MD/PhD
- Dr. Jonathan Trent MD/PhD

Gastrointestinal Stromal Tumor

Understanding Your GIST Pathology Report

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