Diagnosing and Predicting the Behavior of GI Stromal Tumors

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GI Stromal Tumor

- Comprise only 0.2% of all GI tumors, but 80% of GI sarcomas
- 5000 – 6000 new cases per year in the U.S.

### Distribution

- **Stomach**: 60%
- **Small intestine**: 25%
- **Colon/Rectum**: 5%
- **Other (mesentery, retroperitoneum)**: 8%
- **Esophagus**: 2%
Tumor Biopsy

Endoscopic Biopsy

Specimen sent to Pathology lab in formalin
Standard (Rotary) Microtome
For sectioning paraffin-embedded tissues
Sectioning a paraffin-embedded biopsy
Ribbon of biopsy sections floated on waterbath
Like GISTs, ICC cells:
  - express KIT
  - express DOG1
  - express ETV1
Mouse Model Of GIST

Sommer et al., PNAS 100: 6706-11, 2003
Micro-GISTs

- GISTs < 1 cm are common in the general population
  - 10-30% in the stomach
  - <0.2% in the colon & appendix

- Compared with larger GISTs, micro-GISTs have:
  - Similar KIT mutations
  - Lower mitotic index
  - Benign morphology

Rossi et al. 34:1480-1491, 2010
GIST Progression

ICC Cells → Micro-GIST → Low risk GIST → Malignant GIST

- >10 Million
- ~5,000 / yr
- ~2,000 / yr

KIT or PDGFRA mutation → Loss of 14q, 22q MAX → Loss of 1p CDKN2A, RB1, TP53
**KIT and PDGFRA Mutations in GIST**

‘Wild-type’ tumors: 15%

**KIT (75%)**
- Exon 9 (8%)
- Exon 11 (65%)
- Exon 13 (1%)
- Exon 17 (1%)

**PDGFRA (10%)**
- Exon 12 (2%)
- Exon 14 (rare)
- Exon 18 (8%)
  (Includes D842V)
Baseliner                  24 hours           7 days            2 months         5.5 months

Courtesy of Dr. Annick van den Abbele, DFCI

Intracellular pathway:

- KIT / PDGFRA
- SHC
- GRB2
- SOS
- RAS
- RAF
- MEK
- ERK
- STAT

Phosphorylation sites:

- P38
- PDK
- AKT
- S6K
- PTEN
- mTOR

Gene expression changes:

- ↑ JUN, ↑ ETV1, ↑ CDK4, ↑ Cyclin D1, ↓ p16
Genetic Alterations in Wild-type GISTs

- NTRK
- RAS
- BRAF
- MEK
- ERK
- Succinate
- SDHA
- SDHB
- SDHC
- SDHD
- ETV1
- HIF1α
- VEGF, IGF1, IGF2

Prolyl hydroxylase
DNA demethylation
Molecular Subtypes of GIST

- KIT exon 11
- KIT exon 9
- KIT exon 13
- KIT exon 17
- PDGFRA exon 12
- PDGFRA exon 14
- PDGFRA exon 18
- SDHA/B/C/D Mutation
- SDHC methylation
- NF1
- RAS
- NTRK fusion
- BRAF
During a 2 year period, 115 GISTs were diagnosed in the Rhone Alps region of France.

Among these:
- 88% had not spread
  - 36.5% were low or very low risk
  - 35.6% were intermediate risk
  - 27.7% were high risk
- 12% were metastatic
**GIST Management**

- Tumor size
- Tumor location
- Mitoses per 5 mm²

**Low risk of recurrence**

**High risk of recurrence or metastatic**

Treat with imatinib?

- KIT exon 11 mutation → 400 mg imatinib
- KIT exon 9 mutation → 800 mg imatinib
- PDGFRA D842V → no imatinib (clinical trial)

SDHB Stain

CD117+

DOG1+

Imatinib → No
## Primary GIST – Risk of Recurrence

<table>
<thead>
<tr>
<th>Size</th>
<th>Mitotic ≤ 2 cm</th>
<th>Mitotic &gt; 2 ≤ 5 cm</th>
<th>Mitotic &gt; 5 per 5 mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric (n=1055)</td>
<td>0%</td>
<td>1.9%</td>
<td>3.6%</td>
</tr>
<tr>
<td>Jejunum/Ileum (n=629)</td>
<td>0%</td>
<td>4.3%</td>
<td>24%</td>
</tr>
<tr>
<td>Duodenum (n=144)</td>
<td>0%</td>
<td>8.3%</td>
<td>Insuff. data</td>
</tr>
<tr>
<td>Rectum (n=111)</td>
<td>0%</td>
<td>8.5%</td>
<td>Insuff. data</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Size</th>
<th>≤ 2 cm</th>
<th>&gt; 2 ≤ 5 cm</th>
<th>&gt; 5 ≤ 10 cm</th>
<th>&gt; 10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric (n=1055)</td>
<td>(None)</td>
<td>(High)</td>
<td>Insuff. data</td>
<td>54%</td>
</tr>
<tr>
<td>Jejunum/Ileum (n=629)</td>
<td>16%</td>
<td>73%</td>
<td>50%</td>
<td>52%</td>
</tr>
<tr>
<td>Duodenum (n=144)</td>
<td>10%</td>
<td>52%</td>
<td>34%</td>
<td>57%</td>
</tr>
<tr>
<td>Rectum (n=111)</td>
<td>10%</td>
<td>55%</td>
<td>86%</td>
<td>71%</td>
</tr>
</tbody>
</table>

Miettinen & Lasota, Semin Diagn Pathol, 23(2):70-83, 2006
Selecting Tumor-Rich Material for DNA Extraction

Coring a block

Scraping slides
Looking for Mutations: DNA Sequencing

ZITS — By Jerry Scott and Jim Borgman

SO, CAN I GO TO THE LAKE?  LET ME SEE WHAT YOUR DAD THINKS.
Next-Generation DNA Sequencing

• Massively parallel sequencing (many sequencing reactions performed simultaneously)
**Bible Replication Errors**

12 * Honour thy father and thy mother, thy days may long upon the land which LORD thy God giueth thee.
13 * Thou shalt not kill.
14 * Thou shalt commit adultery.
15 Thou shalt not steal.
16 Thou shalt not bear false witness against thy neighbour.
17 * Thou shalt not covet thy neighbour's house or his man-servant or his maid-servant or his ox or his ass or his anything that is thy neighbour's.

**GIST Genome Errors**

**KIT Gene Mutation**

[Pro Tyr Val His Lys]

CCT TAT GTT CAC AAA

CCT TAT --- CAC AAA

[Pro Tyr --- His Lys]

Activated KIT leads to development of GI stromal tumors

**1632 Edition**

*The 'Wicked Bible'*
Bible Replication Errors

1795 Edition  Mark 7:27

'Let the children first be filled'

'Let the children first be killed'

GIST Genome Errors

KIT Gene Mutation

[Ala Thr Val Lys Ser]
GCT ACA GTT AAA TCT

GCT ACA GAG AAA TCT

[Ala Thr Asp Lys Ser]
Summary

- GISTs are a family of tumors arising from mutations in a number of different genes.
- Most GISTs probably arise from ‘micro-GISTs’ through acquisition of mutations or other genetic alterations beyond KIT/PDGFRA/SDH.
- Next-gen sequencing is helpful in molecularly subtyping GISTs.
- Mitotic index, tumor size and tumor location are the 3 most important factors in determining the likelihood of disease recurrence.
Predictive Value of Kinase Genotype In Metastatic GIST Patients On Imatinib

- Exon 11-mutant tumors:
  - Better progression-free and overall survival compared to exon 9 and WT tumors
  - 400 mg is adequate dose
- Exon 9-mutant tumors:
  - Improved progression-free survival when treated with 800 mg imatinib
- PDGFRA D842V-mutant tumors:
  - Resistant
SDH-Deficient GISTs

- Deficient in an enzyme called succinate dehydrogenase
- Nearly always gastric origin
- Multi-nodular growth pattern
- Low mitotic rate, but high rate of recurrence and metastasis
- Poor response to imatinib
# Molecular Classification of GISTs

<table>
<thead>
<tr>
<th>Genetic type</th>
<th>Relative Frequency</th>
<th>Anatomic Distribution</th>
<th>Germline Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KIT Mutation</strong></td>
<td>75%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 8</td>
<td>Rare</td>
<td>Small bowel</td>
<td>1 Kindred</td>
</tr>
<tr>
<td>Exon 9 (insertion 502-503AY)</td>
<td>8%</td>
<td>Small bowel, colon</td>
<td>None</td>
</tr>
<tr>
<td>Exon 11 (deletions, single nucleotide substitutions, insertions)</td>
<td>65%</td>
<td>All sites</td>
<td>Several kindreds</td>
</tr>
<tr>
<td>Exon 13 (K642E)</td>
<td>1%</td>
<td>All sites</td>
<td>3 Kindreds</td>
</tr>
<tr>
<td>Exon 17 (D820Y, N822K, Y823D)</td>
<td>1%</td>
<td>All sites</td>
<td>Several kindreds</td>
</tr>
<tr>
<td><strong>PDGFRA Mutation</strong></td>
<td>10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 12 (deletions, single nucleotide substitutions, insertions)</td>
<td>1%</td>
<td>All sites</td>
<td>2 Kindreds</td>
</tr>
<tr>
<td>Exon 14 (N659K)</td>
<td>Rare</td>
<td>Stomach</td>
<td>None</td>
</tr>
<tr>
<td>Exon 18 D842V</td>
<td>6%</td>
<td>Stomach, mesentery, omentum</td>
<td>None</td>
</tr>
<tr>
<td>Exon 18 (deletions)</td>
<td>2%</td>
<td>All sites</td>
<td>1 Kindred</td>
</tr>
</tbody>
</table>
SDH-Deficient GIST

• Most are due to mutations in SDHA, SDHB, SDHC or SDHD
  – At least half of these mutations are germline
  – Propensity to develop:
    • Paraganglioma and GIST (Carney-Stratakis syndrome)
    • Pancreatic neuroendocrine tumor
    • Renal cell carcinoma, Pituitary adenoma
  – Penetrance varies, even among family members