Personalizing Therapy for GIST (GastroIntestinal Stromal Tumor): c-KIT and PDGFRA Roadmap for Activating and Resistance Mutations

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1st Annual CONPO (Controversies in Personalized Oncology)
Barcelona, Spain, March 2013
ANCIENT HISTORY: Two Different Patients with “Abdominal Leiomyosarcoma” in 1992

Leiomyosarcoma

Gastrointestinal Stromal Tumor (GIST)
Hundreds Of Different Sarcoma Subtypes
What clinical relevance is there to diagnostic categories?

• Does sophisticated molecular profiling of sarcomas improve the discovery, development or choice of therapy?
  – for the right type of cancer
  – in the right patient
  – at the right time in the disease
  – at the right price for individuals and a sustainable society
Gain-of-Function Mutations of c-kit in Human Gastrointestinal Stromal Tumors

Seiichi Hirota,* Koji Isozaki,* Yasuhiro Moriyama, Koji Hashimoto, Toshirou Nishida, Shingo Ishiguro, Kiyoshi Kawano, Masato Hanada, Akihiko Kurata, Masashi Takeda, Ghulam Muhammad Tunio, Yuji Matsuzawa, Yuzuru Kanakura, Yasuhisa Shinomura. Yukihiko Kitamura†

Science 279:577-580, 1998
Validating KIT as a Therapeutic Target in GIST


KIT protein is not affected

ACTIVATED KIT disappears quickly

Hours After Imatinib
0 1 9 24 48
Functional Validation of KIT inhibition with Imatinib

GIST cells grow as control

GIST cells Stop Growing with Imatinib

There was no effective therapy for Metastatic GIST before Kinase Inhibitors

- Chemotherapy never worked
- Only very short survival from first metastasis
- This was a definite unmet medical need
The First U.S. GIST Patient Treated with Imatinib: Dana-Farber Cancer Institute and Harvard Medical School

Baseline

Active Tumor
# Imatinib Benefits the Majority of Patients with Metastatic GIST

<table>
<thead>
<tr>
<th>Best Response</th>
<th>All doses</th>
<th>N = 147 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partial Response</td>
<td></td>
<td>97 (66%)</td>
</tr>
<tr>
<td>Stable Disease</td>
<td></td>
<td>25 (17%)</td>
</tr>
<tr>
<td>Progression or Non-evaluable</td>
<td></td>
<td>25 (17%)</td>
</tr>
</tbody>
</table>

Take Home Points

• Defining GIST by the molecular driver is important

• A high fidelity model is worth its weight in gold

• Clinical diagnosis of GIST vs. LEIOMYOSARCOMA is life-saving
Clinical Detection of KIT (CD117) with IHC

H+E Stain

CD117 IHC Stain

Normal Small Intestine

CDM Fletcher, MD
GIST is the most common subtype of all sarcomas

- GIST: 18%
- Liposarcoma: 11%
- Soft-Tissue Ewing sarcoma/PNET: 11%
- Kaposi sarcoma: 15%
- Dermatofibrosarcoma: 16%
- Unclassified sarcoma: 5%
- Leiomyosarcoma: 3%
- Rhabdomyosarcoma: 7%
- Angiosarcoma: 4%
- Myxofibrosarcoma: 3%
- Synovial sarcoma: 2%
- Endometrial stromal sarcoma: 2%
- Other very rare subtypes: 3%

• Complete responses are very rare

• Why are remaining tumor cells viable, and why is the shape of the tumor preserved?

• This is a form of drug resistance
1000% improvement in overall survival for metastatic GIST treated with imatinib

Study B2222 Team
J Clin Oncol. 2008;26:620-625
Learning from Drug Failure

- Resistance occurs
- Immediately (“primary”) in few
- Delayed (“acquired”) in 90% of patients
Primary Resistance to Imatinib in GIST

5 July 2000

18 September 2000
MUTATIONS DIFFER in GIST with DIFFERENT STRUCTURAL VARIANTS of the Driver Kinase (KIT or PDGFRA)

**KIT**
1 Dominant Mutation per patient – Site of mutation differs between patients

- Exon 9 (8%) - SENS
- Exon 11 (76%) - SENSITIVE
- Exon 13 (1%) - +/-SENS
- Exon 17 (1%) - RES

**PDGFRA**

No mutations in both KIT and PDGFRA (13% wild type) – RESISTANT

- Exon 12 (0.3%) - SENS
- Exon 18 (0.6%) - RES

Cell Membrane

Cytoplasm
GIST with different mutations behave differently

Different Genotypic and Structural Variants Fail Imatinib Therapy at Different Rates

Heinrich, Corless, Fletcher, Demetri et al.
Pediatric-Type GIST has NO KINASE MUTATIONS!

SNP LOH display shows few LOH regions in KIT–wild-type pediatric GISTs compared with KIT-mutant pediatric or adult GISTs. Cases in bold have companion normal DNAs. Blue, LOH; yellow, retained heterozygosity.

Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations

Katherine A. Janeway*,1,2, Su Young Kim*,1, Maya Lodish*, Vânia Nosé, Pierre Rustin*, José Gaal†, Patricia L. M. Dahia‡, Bernadette Liegl*, Evan R. Ball‡, Margarita Raygada*, Angela H. Lai*, Lorna Kelly*, Jason L. Hornick*, NIH Pediatric and Wild-Type GIST Clinic,1,4,5,6,7,8,9 Maureen O’Sullivan,1,5,6,7,8,9 Ronald R. de Krijger,1,5,6,7,8,9 Winand N. M. Dinjens,1,5,6,7,8,9 George D. Demetri†, Cristina R. Antonescu*, Jonathan A. Fletcher‡, Lee Helman*, and Constantine A. Stratakis*
GIST is one histopathologic cancer with several distinct molecular drivers

<table>
<thead>
<tr>
<th><strong>KIT mutant</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 11</td>
<td>Most common site of mutation (67%)</td>
</tr>
<tr>
<td>Exon 9</td>
<td>2(^{nd}) most common site of mutation (10%)</td>
</tr>
<tr>
<td>Exons 13 &amp; 17</td>
<td>Rare (2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>PDGFRA mutant</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Exons 12 &amp; 14</td>
<td>Rare (1%)</td>
</tr>
<tr>
<td>Exon 18</td>
<td>Uncommon (6%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>BRAF mutant</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>“Wild-type”</td>
<td>Exceptionally rare (&lt;1%)</td>
</tr>
</tbody>
</table>

| **“Wild-type”** | No mutation in KIT or PDGFRA: SDH(x) deficient in general (14%) |

<table>
<thead>
<tr>
<th><strong>Familial GIST</strong></th>
<th>Germline KIT or PDGFRA mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pediatric</strong></td>
<td>KIT &amp; PDGFRA are generally “Wild type” (no mutation)</td>
</tr>
<tr>
<td>Carney’s triad</td>
<td>KIT &amp; PDGFRA are generally “Wild type” (no mutation)</td>
</tr>
<tr>
<td>Carney-Stratakis</td>
<td>Mutations in metabolism enzymes (SDH subunit loss in A,B,C or D) causes functional loss</td>
</tr>
<tr>
<td><strong>NF-1-related</strong></td>
<td>KIT &amp; PDGFRA are generally “Wild type” (no mutation)</td>
</tr>
</tbody>
</table>
Patients Identify with Molecular Medicine
Should GIST Mutation Drive Clinical Decision-Making?

- Can we treat GIST patients more precisely by using genotype information?
- Does it make a clinical difference?
**Meta-Analysis of Progression-Free Survival: Does Imatinib DOSE matter?**

- **Median PFS (months):** 19 / 23
- **3-year estimate (%):** 30 / 34
- **Hazard ratio:** 0.89
- **P value (logrank test):** 0.04

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Van Glabbeke et al., ASCO 2007, Abstract #10004

- **400 mg (818 patients)**
- **800 mg (822 patients)**
Meta-Analysis of Progression-Free Survival by GIST MUTATIONAL SUBTYPE (exon 9 vs. others)

<table>
<thead>
<tr>
<th>KIT exon 9 mutants</th>
<th>Median PFS (months)</th>
<th>6 / 19</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-year estimate (%)</td>
<td>5 / 17</td>
</tr>
<tr>
<td></td>
<td>P value (logrank test)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

KIT exon 9 mutants: 400 mg / 800 mg
Other patients: 400 mg / 800 mg

Van Glabbeke et. al., ASCO 2007, Abstract #10004
Adjuvant Systemic Therapy for Primary Localized GIST

Proper decision-making requires understanding of RISK OF RELAPSE from the primary localized GIST

Key Factors that drive risk of recurrence in the GIST PRIMARY:

- **SIZE**
- Number of **MITOSES**
- ANATOMIC SITE of PRIMARY
- GENOTYPE
ADJUVANT Imatinib vs. Placebo Phase III Trial: Primary Resected GIST

Double-Blind, Placebo-Controlled Clinical Trial: NCI-funded ACOSOG Z9001

Randomization

PRIMARY GIST > 3 CM

(IMATINIB)

400 mg/day x 1 year

IMATINIB

Placebo x 1 year

Primary Endpoint: RECURRENCE-FREE SURVIVAL

Recurrence-Free Survival
ACOSOG Z9001

GIST Adjuvant Benefit Correlates with Tumor Genotype

NO IMATINIB BENEFIT IN RFS for GIST without KIT or PDGFRA Mutations ("Wild Type" GIST)

\[ p = 0.6126 \text{ at 24 months} \]

Corless et al. ASCO 2010
GIST Adjuvant Benefit Correlates with Tumor Genotype

NO IMATINIB BENEFIT IN RFS for GIST with PDGFRA D842V Mutation

\[ p = 0.9984 \]

Corless et al ASCO 2010
KIT Activation Is Rapidly Inhibited in GIST Patients Receiving Imatinib Treatment – but REACTIVATES with Progression
Emergence of New Secondary Mutations in \textit{KIT} Can Explain Resistance to Imatinib in GIST
Response in GIST Followed By SECONDARY Resistance

1st Resistance Mutation

2nd Resistance Mutation

3rd Resistance Mutation

1 month on Imatinib

9 MONTHS on Imatinib
Is It Possible to Develop “Culture and Sensitivity” Assays for GIST?

• We perform “culture and sensitivity” testing on pathogenic bacterial isolates

• We assay retroviral genetic factors which assist in HIV patient management

• Cancer management has traditionally been relatively empiric rather than mechanistic
Understanding resistance to overcome the problem

• Biological
  – Double mutants MUST have an evolutionary DISadvantage and be “less fit”
  – Double mutants only evolve to clinically detectable levels after single mutants are suppressed with imatinib

• Structural
Imatinib vs. Sunitinib: Profiling the Kinome

In-Vitro Effects of Sunitinib or Imatinib on KIT DOUBLE Mutants: Exons 11 + 13

Exon 11 + Exon 13
V560 + V654D

Imatinib (µM) 0 10 5 1 0.1
pKIT
KIT

Sunitinib (µM) 0 2 1 0.5 0.1
pKIT
KIT

pKIT = phosphorylated KIT

In-Vitro Effects of Sunitinib or Imatinib on KIT DOUBLE Mutants: Exons 11 + 13

Exon 11 + Exon 13
V560 + V654D

<table>
<thead>
<tr>
<th>Imatinib (µM)</th>
<th>0</th>
<th>10</th>
<th>5</th>
<th>1</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKIT</td>
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<tr>
<td>KIT</td>
<td></td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Sunitinib (µM)</th>
<th>0</th>
<th>2</th>
<th>1</th>
<th>0.5</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKIT</td>
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<tr>
<td>KIT</td>
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</tbody>
</table>

Signal ON (resistant to Imatinib)

pKIT = phosphorylated KIT

**In-Vitro** Effects of Sunitinib or Imatinib on **KIT** DOUBLE Mutants: Exons 11 + 13

- **Exon 11 + Exon 13**
  - V560 + V654D

<table>
<thead>
<tr>
<th>Imatinib (μM)</th>
<th>0</th>
<th>10</th>
<th>5</th>
<th>1</th>
<th>0.1</th>
</tr>
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<tbody>
<tr>
<td>pKIT</td>
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<td></td>
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<tr>
<td>KIT</td>
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</table>

<table>
<thead>
<tr>
<th>Sunitinib (μM)</th>
<th>0</th>
<th>2</th>
<th>1</th>
<th>0.5</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>pKIT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIT</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

- **Signal ON** (resistant to Imatinib)
- **Signal OFF** (resistant to Imatinib BUT sensitive to Sunitinib)

pKIT = phosphorylated KIT

In-Vitro Effects of Sunitinib or Imatinib on KIT DOUBLE Mutants: Exons 11 + 13 vs. Exons 11 + 17

Exon 11 + Exon 13
V560 + V654D

Exon 11 + Exon 17
V560D + Y823D

pKIT = phosphorylated KIT

**In-Vitro Effects of Sunitinib and Imatinib on KIT Mutants: Summary**

<table>
<thead>
<tr>
<th>Mutation(s)</th>
<th>Exon(s)</th>
<th>Location of second mutation</th>
<th>Approximate IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V560D</td>
<td>11</td>
<td>–</td>
<td>Sunitinib: &lt;50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imatinib: ~100</td>
</tr>
<tr>
<td>V654A</td>
<td>13</td>
<td>–</td>
<td>Sunitinib: &lt;50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imatinib: 5000</td>
</tr>
<tr>
<td>V560D + V654A</td>
<td>11 + 13</td>
<td>ATP BP</td>
<td>Sunitinib: &lt;100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imatinib: 5000</td>
</tr>
<tr>
<td>V560D + T670I</td>
<td>11 + 14</td>
<td>ATP BP</td>
<td>Sunitinib: &lt;50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imatinib: 10,000</td>
</tr>
<tr>
<td>V560D + D816H</td>
<td>11 + 17</td>
<td>Act. loop</td>
<td>Sunitinib: 1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imatinib: 5000</td>
</tr>
<tr>
<td>V560D + N822K</td>
<td>11 + 17</td>
<td>Act. loop</td>
<td>Sunitinib: &gt;1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imatinib: ~1000</td>
</tr>
<tr>
<td>V560D + V823D</td>
<td>11 + 17</td>
<td>Act. loop</td>
<td>Sunitinib: &gt;1000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imatinib: &gt;1000</td>
</tr>
<tr>
<td>Exon 9 + V654A</td>
<td>9 + 13</td>
<td>ATP BP</td>
<td>Sunitinib: ~100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Imatinib: 5000</td>
</tr>
</tbody>
</table>

Act. loop = activation loop  
ATP BP = ATP binding pocket

**SENSITIVE vs. RESISTANT**

Rational Approach to Drug Resistance: Sunitinib, a structurally distinct kinase inhibitor

Baseline

PET scan after 7 days of Sunitinib
Normal heart
Normal kidneys

CT after 2 months of Sunitinib

Sunitinib Improves Progression-Free Survival in GIST following failure of Imatinib

Sunitinib (N=207)  Placebo (N=105)
Median TTP (95% CI) 6.3 mo (3.7-7.6) 1.5 mo (1.0-2.3)

Hazard ratio = 0.335  P<0.00001

Structural explanation for why Sunitinib works in Imatinib-resistant GIST

K Gajiwala, Pfizer Oncology and G Demetri, Dana-Farber/Harvard

1Gajiwala et al. Proc Natl Acad Sci USA 2009;106:1542
Multiclonal Resistance = Multifocal Progression

<table>
<thead>
<tr>
<th>Patient type</th>
<th>Patients (n)</th>
<th>Tumors (n)</th>
<th>Samples (n)</th>
</tr>
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<tbody>
<tr>
<td>TKI-treated progressing GIST</td>
<td>1</td>
<td>46</td>
<td>52</td>
</tr>
</tbody>
</table>
# Multiclonal Resistance Mutations

<table>
<thead>
<tr>
<th></th>
<th>KIT 1&lt;sup&gt;st&lt;/sup&gt; mutation</th>
<th>KIT 2&lt;sup&gt;nd&lt;/sup&gt; mutation</th>
<th>KIT 1&lt;sup&gt;st&lt;/sup&gt; mutation</th>
<th>KIT 2&lt;sup&gt;nd&lt;/sup&gt; mutation</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Ex 9</td>
<td>Q575_P577delinsH</td>
<td>17</td>
<td>Ex 9</td>
</tr>
<tr>
<td>2</td>
<td>Ex 9</td>
<td>S840N</td>
<td>18a</td>
<td>Ex 9</td>
</tr>
<tr>
<td>3</td>
<td>Ex 9</td>
<td>S840N</td>
<td>18b</td>
<td>Ex 9</td>
</tr>
<tr>
<td>4</td>
<td>Ex 9</td>
<td>18c</td>
<td>Ex 9</td>
<td>S840N</td>
</tr>
<tr>
<td>5</td>
<td>Ex 9</td>
<td>19</td>
<td>Ex 9</td>
<td>N822K</td>
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<tr>
<td>6</td>
<td>Ex 9</td>
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<td>Ex 9</td>
</tr>
<tr>
<td>7</td>
<td>Ex 9</td>
<td>I571_D572delinsT</td>
<td>21</td>
<td>Ex 9</td>
</tr>
<tr>
<td>8a</td>
<td>Ex 9</td>
<td>S840N</td>
<td>22</td>
<td>Ex 9</td>
</tr>
<tr>
<td>8b</td>
<td>Ex 9</td>
<td>S840N</td>
<td>23</td>
<td>Ex 9</td>
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<td>9</td>
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<td>10</td>
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</tr>
<tr>
<td>46</td>
<td>Ex 9</td>
<td>N822K</td>
<td>46</td>
<td>Ex 9</td>
</tr>
</tbody>
</table>

Courtesy of Yuexiang Wang (Fletcher lab, BWH)
Oral Kinase Inhibitors of Multiple Kinases Relevant to GIST and Other Cancers

Regorafenib in Patients with Metastatic GIST following failure of Imatinib and Sunitinib
Regorafenib in GIST: Phase 3 Improvement in Progression-Free Survival

- Regorafenib significantly improved PFS vs placebo (p<0.0001); primary endpoint met

Demetri G, et al. LANCET 2012 epub online

<table>
<thead>
<tr>
<th></th>
<th>Regorafenib, N=133</th>
<th>Placebo, N=66</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median PFS</td>
<td>4.8 months (4.1–5.8)</td>
<td>0.9 months (0.9–1.1)</td>
</tr>
<tr>
<td>Number of events</td>
<td>81 (60.9%)</td>
<td>63 (95.5%)</td>
</tr>
<tr>
<td>Hazard ratio (95% CI)</td>
<td>0.27 (0.19–0.39)</td>
<td></td>
</tr>
<tr>
<td>1-sided p-value</td>
<td>&lt;0.0001</td>
<td></td>
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</table>
Patients are smarter than ever.

New networking tools allow better access to information and clinical trials.

Molecular and genomic diagnostics with functional screens and validations can change the way clinical research is conducted.
There's a whole new world inside. Discover it now.

I'm a [ ] looking for [ ]

Between ages [ ] and [ ]

Near ZIP/Postal code [ ]

GET THE 6 MONTH GUARANTEE. [GO]
Thanks to All the Collaborators and Patients

- Clinical Teams at Dana-Farber/Harvard Cancer Center
  - Suzanne George, James Butrynski, Andy Wagner, Katie Janeway, Jeff Morgan, A Potter, K Polson, J Field
- Molecular Biology and Molecular Pathology
  - David Tuveson, Michael Heinrich, Jonathan Fletcher
  - Yossi Schlessinger, Julie Cherrington, Peter Hirth, Brian Druker
- Immunohistochemistry and Anatomic Pathology
  - Christopher Fletcher, Chris Corless
- Surgical Oncology
  - Chan Raut, Monica Bertagnolli, Burt Eisenberg, Peter Roberts
- Imaging; Statistics
  - Annick van den Abbeele; Judith Manola
- Clinical Teams (US-Finland: C. Blanke, M. von Mehren, H. Joensuu)
  - EORTC: A. van Oosterom, J. Verweij, A. LeCesne, I. Judson, JY Blay
  - Australia: J. Zalcberg, G. McArthur, J. Desai
- Industry Collaborators
  - Novartis -- Bayer
  - Pfizer -- Blueprint
  - Infinity -- Kolltan
  - Roche/Genentech