Genomics of “Wild-Type” GIST: Domestication In Progress

Jason Sicklick, MD
Associate Professor of Surgery
Genomics 101

23 + 23 = 46 chromosomes
Cancer Is A Disease Of The Genome

- DNA is exposed to carcinogenic events every day; this causes gene alterations to occur.
- Exposure to cancer risk factors increases the chances of gene alterations.

UC San Diego
Moores Cancer Center
How Gene Alterations Can Cause Cancer

**ALTERNED GENES**

Code for

**ALTERNED PROTEINS**

Resulting in

**ALTERNED PATHWAYS**

- RAS
- RAF
- MAPK

**CANCER**
Of the ~20,000 genes in the genome, only a subset of a few hundred are unambiguously associated with cancer.

Gene names:

- KIT
- PDGFRA
- BRAF
- KRAS
- HRAS
- NRAS
- FGFR1
Types Of Alterations In Cancer Genes

- Rearrangements
- Fusions
- Substitutions (Missense)
- Copy number alterations
- Insertions and deletions

Normal
The Shift Toward Targeted Therapy

Chemotherapy

- Anticancer drugs may be highly effective in some, but less effective in others
- Patients encounter side effects which are often significant

Targeted Therapy

- In personalized medicine, clinicians use biomarkers to predict a patient's response to therapy
- Patients are more likely to get therapies with the greatest impact which often have fewer side effects
Advantages Of Comprehensive Genomic Profiling (CPG) vs. Traditional Hot Spot Testing

**Hot Spot or Single-Marker Testing**
- Misses some types of mutations (rearrangements/fusions, copy number alterations)
- Limited number of alterations screened at once
- Results are specific for the test used: need to know ahead of time what questions to ask
- Exhausts tissue

**CGP**
- Able to identify hundreds of clinically relevant mutations at once
- Allows the opportunity to identify all alterations
- Tissue sparing
### Alterations Detected

<table>
<thead>
<tr>
<th>Normal</th>
<th>CGP</th>
<th>Hot Spot</th>
</tr>
</thead>
</table>

**Substitutions Missense**
- Normal: Green check mark
- CGP: Green check mark
- Hot Spot: Red x

**Copy number alterations**
- Normal: Green check mark
- CGP: Green check mark
- Hot Spot: Red x

**Insertions and deletions**
- Normal: Green check mark
- CGP: Green check mark
- Hot Spot: Red x

**Rearrangements Fusions**
- Normal: Green check mark
- CGP: Green check mark
- Hot Spot: Red x
Cancer Related Genes in GIST

<table>
<thead>
<tr>
<th>Gene names</th>
</tr>
</thead>
<tbody>
<tr>
<td>KIT</td>
</tr>
<tr>
<td>PDGFRA</td>
</tr>
<tr>
<td>BRAF</td>
</tr>
<tr>
<td>KRAS</td>
</tr>
<tr>
<td>HRAS</td>
</tr>
<tr>
<td>NRAS</td>
</tr>
<tr>
<td>FGFR1</td>
</tr>
</tbody>
</table>
Known Driver Genes in 85-90% of GIST

Pantaleo et al., Cancer Medicine. 2015.
Lack Mutations in \textit{KIT, PDGFRA, RAS Pathway (NF1, RAS, BRAF)} and \textit{SDH Subunits}

\section*{Quadruple Wild-type (qWT) GIST}

- Genomics?
- Epidemiology?
- Disease Biology?

\begin{itemize}
  \item SDHB+
  \item IGF1R-
  \item Any age?
  \item Equal sex?
  \item Site?
  \item Multifocality?
\end{itemize}

\textit{Pantaleo et al., Cancer Medicine. 2015.}
Hypothesis

Broad genomic profiling of “quadruple-WT (qWT)” GISTs would reveal insights into the genomic alterations and disease biology of this understudied patient population.
Methods

Patient Population and Data Collection

- Foundation Medicine, Inc. (FMI) database consisting of de-identified patients from across the U.S. (October 2012 – May 2015).
- Retrospectively analyzed this prospectively collected data.

Broad Genomic Profiling

- DNA was extracted from FFPE tumor specimens.
- NGS assay utilizes the Illumina HiSeq 2500 instrument to sequence against hybridization-captured, adaptor ligation-based libraries for coding regions of 315 cancer-related genes plus introns from 28 genes frequently implicated in cancer transformation.
Methods (Continued)

Data Analysis

• Genomic alterations were further categorized:
  • Known somatic
  • Likely somatic
  • Variant of unknown significance (VUS).

• To understand of the potential deleterious effects of all missense VUS’s, we analyzed them with 4 prediction modeling programs (SIFT, PolypPhen, MutationTaster, and MutationAssessor).

• Considered potentially deleterious if they were predicted deleterious by ≥50% tools. Of 1240 VUS’s, we considered 325 (26.2%) potentially deleterious.

• Exome Aggregation Consortium (ExAC) Browser was used to exclude missense variants with a minor allele frequency >1% (NOTCH2, FANCD2, MAP3K1, MSH3, and ZNF217).
Somatic Genomic Landscape in 186 GIST
Driver Mutations in 186 GIST

- **KIT (N=129)**: 69%
- **PDGFRA (N=22)**: 12%
- **NF1 (N=18)**: 10%
- **SDHx (N=14)**: 8%
- **BRAF (N=7)**: 4%
- **[KHN]-RAS (N=4)**: 2%
- **qWT (N=12)**: 6%
- **tWT*: sequencing performed before FMI testing of SDHx genes**

* tWT* = sequencing performed before FMI testing of SDHx genes
# Demographics of GIST Patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>WT GIST</th>
<th>Non-WT GIST</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Total Patients</td>
<td>24</td>
<td>162</td>
<td></td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>44.4 ± 15.7</td>
<td>58.3 ± 14.1</td>
<td>&lt;0.01</td>
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<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12 (50.0)</td>
<td>66 (40.7)</td>
<td>0.51</td>
</tr>
<tr>
<td>Male</td>
<td>12 (50.0)</td>
<td>94 (58.0)</td>
<td></td>
</tr>
<tr>
<td>Not Reported</td>
<td>-</td>
<td>2 (1.2)</td>
<td></td>
</tr>
<tr>
<td>Primary GIST Site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>2 (8.3)</td>
<td>15 (9.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>Small intestine</td>
<td>9 (37.5)</td>
<td>44 (27.2)</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>13 (54.2)</td>
<td>83 (51.2)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0 (0.0)</td>
<td>20 (12.3)</td>
<td></td>
</tr>
<tr>
<td>Variables</td>
<td>qWT GIST N (%)</td>
<td>tWT GIST N (%)</td>
<td>P-value</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Total Patients</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Age (years, mean ± SD)</td>
<td>44.0 ± 14.9</td>
<td>44.8 ± 17.1</td>
<td>0.90</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>5 (41.7)</td>
<td>7 (58.3)</td>
<td>0.68</td>
</tr>
<tr>
<td>Male</td>
<td>7 (58.3)</td>
<td>5 (41.6)</td>
<td></td>
</tr>
<tr>
<td>Not Reported</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Primary GIST Site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>0 (0.0)</td>
<td>2 (16.7)</td>
<td>0.36</td>
</tr>
<tr>
<td>Small intestine</td>
<td>4 (33.3)</td>
<td>5 (41.6)</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>8 (66.7)</td>
<td>5 (41.6)</td>
<td></td>
</tr>
</tbody>
</table>
## Demographics of GIST Patients

<table>
<thead>
<tr>
<th>TNM Classification</th>
<th>qWT GIST N (%)</th>
<th>tWT GIST N (%)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tumor Size (T)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 (≤ 2 cm)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>T2 (&gt;2, ≤5 cm)</td>
<td>0 (0.0)</td>
<td>2 (16.7)</td>
<td></td>
</tr>
<tr>
<td>T3 (&gt;5, ≤10 cm)</td>
<td>11 (91.7)</td>
<td>5 (41.6)</td>
<td></td>
</tr>
<tr>
<td>T4 (&gt;10 cm)</td>
<td>1 (8.3)</td>
<td>4 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Tx</td>
<td>0 (0.0)</td>
<td>1 (8.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Regional Lymph Nodes (N)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>6 (50.0)</td>
<td>2 (16.7)</td>
<td>0.14</td>
</tr>
<tr>
<td>N1</td>
<td>3 (25.0)</td>
<td>8 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Nx</td>
<td>3 (25.0)</td>
<td>2 (16.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Distant Metastases (M)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>M1</td>
<td>9 (75.0)</td>
<td>8 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Mx</td>
<td>3 (25.0)</td>
<td>4 (33.3)</td>
<td></td>
</tr>
</tbody>
</table>
Types of Genomic Alterations Detected

- Missense (N=120): 67.8%
- In-Frame Indel (N=19): 10.7%
- Frameshift (N=11): 6.2%
- Other (N=10): 5.6%
- Nonsense (N=6): 3.4%
- Fusion (N=5): 2.8%
- Rearrangements (N=3): 1.7%
- Rearrangements (N=3): 1.7%
Heterogeneous Set of Genomic Alterations
(Known/Likely + Potentially Deleterious VUS)

* Include only genes with ≥2 genomic alterations
7 Genes Significantly More Affected

<table>
<thead>
<tr>
<th>Gene</th>
<th>Aliases</th>
<th>Alterations in non-WT (%)</th>
<th>Alterations in WT (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARID1B</td>
<td>AT Rich Interactive Domain 1B</td>
<td>11 (6.79%)</td>
<td>5 (20.83%)</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>FGFR1</strong></td>
<td>Fibroblast growth factor receptor 1</td>
<td>4 (2.47%)</td>
<td>3 (12.5%)</td>
<td>0.047</td>
</tr>
<tr>
<td>ATR</td>
<td>Ataxia telangiectasia and Rad3 related</td>
<td>4 (2.47%)</td>
<td>3 (12.5%)</td>
<td>0.047</td>
</tr>
<tr>
<td>LTK</td>
<td>Lymphocyte receptor tyrosine kinase</td>
<td>2 (1.23%)</td>
<td>3 (12.5%)</td>
<td>0.02</td>
</tr>
<tr>
<td>SUFU</td>
<td>Suppressor of Fused</td>
<td>0 (0%)</td>
<td>2 (8.33%)</td>
<td>0.02</td>
</tr>
<tr>
<td>ZNF217</td>
<td>Zinc Finger 217</td>
<td>0 (0%)</td>
<td>2 (8.33%)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>PARK2</strong></td>
<td>Parkin RBR E3 Ubiquitin Protein Ligase</td>
<td>1 (0.62%)</td>
<td>2 (8.33%)</td>
<td>0.044</td>
</tr>
</tbody>
</table>
FGFR1 Gene Fusions Identified in $\frac{2}{3}^{rd}$ GISTs

![Diagram showing FGFR1 gene fusions in GISTs](image-url)
Gene Fusions

- Hybrid gene formed from 2 previously separate genes
- It can occur as a result of 3 mechanisms:

A. Chromosomal Translocation

Chromosome 1
Chromosome 2
Derivative
Chromosome 2
Gene Fusion

Transfer

Promoter Region (including start codon)
Coding Regions
Stop Codon
Intergenic Space
Break Point
CTOS in November 2015

<table>
<thead>
<tr>
<th>Gene</th>
<th>Fusion</th>
<th>Previously Reports</th>
</tr>
</thead>
<tbody>
<tr>
<td>FGFR1</td>
<td>FGFR1-TACC1</td>
<td>Glioblastoma multiforme</td>
</tr>
<tr>
<td></td>
<td>FGFR1-HOOK3</td>
<td>RET-HOOK3 fusion in papillary thyroid cancer</td>
</tr>
<tr>
<td>ETV6</td>
<td>ETV6-NTRK3</td>
<td>Infantile fibrosarcoma secretory breast carcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>salivary gland tumors</td>
</tr>
</tbody>
</table>

ETV6-NTRK3 in qWT GIST

## OHSU Validation in 2\textsuperscript{nd} Study Population

### Target Kinase | Fusion Partners
--- | ---
AKT3 | MAGI3
ALK | ATIC, C2orf44, CARS, CLTC, EML4, FN1, KIF5B, KLC1, MSN, NPM1, PPFIBP1, PTPN3, SEC31A, SQSTM1, STRN, TFG, TPM3, TPM4, TRAF1, VCL
BRAF | AGK, AGTRAP, AKAP9, CLCN6, FAM131B, FCHSD1, GNAI1, KCTD7, KIAA1549, MAD1L1, MKRN1, NUDEL3, PLIN3, RNFL30, SLC45A3, SOX6, TRIM24, ZKSCAN5
EGFR | EGFR variant III, CAND1, PSPH, SEPT14, SLC12A9
ERBB4 | EZR
ERG | TMPRSS2
FGFR1 | BAG4, CPSF6, ERLIN2, PLAG1, TACC1, ZNF703
FGFR2 | AFF3, AHCYL1, BICC1, CASP7, CCDC6, CIT, KIAA1967, OFD1, SLC45A3
FGFR3 | BAIAP2L1, TACC3
MET | MIR548F1, TPR
NTRK1 | BCAN, CD74, MIR548F1, MPRIP, NFASC, TFG, TPM3, TPR
NTRK2 | NACC2, QKI
NTRK3 | ETV6
NRG1 | CD74, SLC3A2
PDGFRA | KDR, SCAF11
PDGFRB | NIN
RAF1 | DAZL, ESRP1, MSS51, SRGAP3
RET | AFAP1, CCDC6, ERC1, HOOK3, KIAA1468, KIF5B, NCOA4, PARG, PCM1, PRKAR1A, TRIM27, TRIM33
ROS1 | CCDC6, CD74, CEP85L, EZR, GOPC, KDELRI2, LRIG3, SDC4, SLC34A2, TFG, TPM3

Compliments of M. Heinrich and C. Corless, OHSU.
## 5 qWT GIST in OHSU Study Population

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>Gender</th>
<th>Primary Tumor Location</th>
<th>Tumor Stage</th>
<th>SDHB Immunostaining</th>
<th>Fusion Panel Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>Male</td>
<td>Pelvic mass</td>
<td>Unknown</td>
<td>Unknown</td>
<td><strong>FGFR1-TACC1</strong></td>
</tr>
<tr>
<td>54</td>
<td>Male</td>
<td>Colon</td>
<td>Unknown</td>
<td>Positive</td>
<td><strong>ETV6-NTRK3</strong></td>
</tr>
<tr>
<td>49</td>
<td>Male</td>
<td>Small intestine</td>
<td>T3NxMx</td>
<td>Positive</td>
<td>None detected</td>
</tr>
<tr>
<td>51</td>
<td>Female</td>
<td>Unknown</td>
<td>TxN1Mx</td>
<td>Positive</td>
<td>None detected</td>
</tr>
<tr>
<td>53</td>
<td>Male</td>
<td>Stomach</td>
<td>Unknown</td>
<td>Unknown</td>
<td>None detected</td>
</tr>
</tbody>
</table>

**FGFR1**
- Extracellular domain
- Kinase domain
- Exons 2-17

**FGFR1-TACC1**
- Extracellular domain
- Kinase domain
- Coiled-coil domain

**NTRK3**
- Extracellular domain
- Kinase domain
- Exons 1-5

**ETV6-NTRK3**
- PNT domain
- Kinase domain
- Exons 14-19

Compliments of M. Heinrich and C. Corless, OHSU.
ETV6-NTRK3 Sensitizes Cells to IGF1R and ALK Inhibitors

Infantile Fibrosarcoma & Osteosarcoma Cell Lines

IGF-1R inhibitors

ALK inhibitors

Neurotrophic tropomyosin receptor kinase (NTRK)

Amatu et al., ESMO Open. 2016.
### Table 2: Ongoing phase II/III trials involving drugs with known inhibitory activity of NTRK-related kinases

<table>
<thead>
<tr>
<th>NCT/EudraCT number</th>
<th>Title</th>
<th>Drug</th>
<th>Targets</th>
<th>Phases</th>
<th>Patients</th>
<th>Start date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02210711</td>
<td>Phase 1/II study of MOCD616 in patients with advanced cancer</td>
<td>MOCD616</td>
<td>MET, AXL, c-kit, MER, DDR2, VEGFR, PDGFR, RET, Trk, Eph</td>
<td>1</td>
<td>120</td>
<td>August 2014</td>
</tr>
<tr>
<td>NCT02568207</td>
<td>Basket study of entrectinib (RXDX-101) for the treatment of patients with solid tumors harboring NTRK1/2/3, ROS1, or ALK gene rearrangements (fusions)</td>
<td>Entrectinib (RXDX-101)</td>
<td>TrkA, TrkB, TrkC, ROS1, ALK</td>
<td>2</td>
<td>300</td>
<td>October 2015</td>
</tr>
<tr>
<td>NCT02097810</td>
<td>Study of oral RXDX-101 in adult patients with locally advanced or metastatic cancer targeting NTRK1, NTRK2, NTRK3, ROS1, or ALK molecular alterations</td>
<td>RXDX-101</td>
<td>1/2</td>
<td>175</td>
<td>June 2014</td>
<td></td>
</tr>
<tr>
<td>NCT02650401</td>
<td>Study of RXDX-101 in children with recurrent or refractory solid tumors and primary CNS tumors</td>
<td>RXDX-101</td>
<td>TrkA, ALK</td>
<td>1</td>
<td>80</td>
<td>December 2015</td>
</tr>
</tbody>
</table>

*Note: Bolded entries indicate trials specifically focusing on lymphomas.*

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Amatu et al., ESMO Open. 2016.
The efficacy of larotrectinib (LOXO-101), a selective tropomyosin receptor kinase (TRK) inhibitor, in adult and pediatric TRK fusion cancers

Hyman DM, 1 Laetsch TW, 2 Kummar S, 3 DuBois SG, 4 Farago AF, 5 Pappo AS, 6 Demetri GD, 7 El-Deiry WS, 8 Lassen UN, 9 Dowlati A, 10 Brose MS, 11 Boni V, 12 Turpin B, 13 Nagasubramanian R, 14 Cruickshank S, 15 Cox MC, 15 Ku NC, 15 Hawkins DS, 16 Hong DS, 17 Drilon AE 1

1Memorial Sloan Kettering Cancer Center, New York, NY; 2University of Texas Southwestern, Dallas, TX; 3Stanford University School of Medicine, Palo Alto, CA; 4Dana-Farber Cancer Institute/Boston Children’s Cancer and Blood Disorders Center, Boston, MA; 5Massachusetts General Hospital, Boston, MA; 6St. Jude Children’s Research Hospital, Memphis, TN; 7Dana-Farber Cancer Institute/Harvard Medical School, Boston, MA; 8Fox Chase Cancer Center, Philadelphia, PA; 9Rigshospitalet, Copenhagen, Denmark; 10UH Cleveland Medical Center, Cleveland, OH; 11Department of Otorhinolaryngology: Head and Neck Surgery, Abramson Cancer Center of the University of Pennsylvania, Philadelphia, PA; 12START Madrid CIOCC, Hospital HM Universitario Sanchinarro, Madrid, Spain; 13Cincinnati Children’s Hospital Medical Center, Cincinnati, OH; 14Nemour’s Children’s Hospital, Orlando, FL; 15Loxo Oncology, Inc., San Francisco, CA; 16Seattle Children’s Hospital, University of Washington, Fred Hutchinson Cancer Research Center, Seattle, WA; 17The University of Texas MD Anderson Cancer Center, Houston, TX.
Treatment Refractory *ETV6-NTRK3* GIST

**Baseline**

**Week 8**

LOXO-101

*Larotrectinib*

Shi et al., *JTM* 2016.
Diversity of cancers treated - 17 unique types

- Peripheral nerve sheath tumor: 4%
- Sarcoma, NOS: 4%
- Myopericytoma: 4%
- Cholangiocarcinoma: 4%
- Spindle cell sarcoma: 5%
- GIST: 5%
- Melanoma: 7%
- Lung: 7%
- Colon: 7%
- Infantile fibrosarcoma (IFS): 13%
- Thyroid: 9%
- Inflammatoty myofibroblastic kidney tumor: 2%
- Salivary gland: 22%
- Infantile myofibromatosis: 2%
- Pancreatic: 2%
- Breast: 2%
- Appendix: 2%
# Efficacy of larotrectinib in TRK fusion cancers

<table>
<thead>
<tr>
<th>Enrolled Patients with Confirmatory Response Data Available (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objective Response Rate</strong> (ORR = PR + CR)</td>
</tr>
<tr>
<td>76%</td>
</tr>
<tr>
<td>(95% CI: 62% – 87%)</td>
</tr>
<tr>
<td><strong>Partial Response (PR)</strong></td>
</tr>
<tr>
<td>64%</td>
</tr>
<tr>
<td><strong>Complete Response (CR)</strong></td>
</tr>
<tr>
<td>12%</td>
</tr>
<tr>
<td><strong>Stable Disease</strong></td>
</tr>
<tr>
<td>12%</td>
</tr>
<tr>
<td><strong>Progressive Disease</strong></td>
</tr>
<tr>
<td>12%</td>
</tr>
</tbody>
</table>

![Graph](image)

*Patient had TRK solvent front resistance mutation (NTRK3 G623R) at baseline due to prior therapy; *Pathologic CR.

Note: One patient not shown here. Patient experienced clinical progression and no post-baseline tumor measurements were recorded.
FGFR1 Gene Fusions Identified GIST
Fibroblast Growth Factor Receptor 1 (FGFR1)

Amatu et al., ESMO Open. 2016. 
GSI Website
Lack Mutations in *KIT*, *PDGFRA*, RAS Pathway (*NF1*, *RAS*, *BRAF*) and SDH Subunits

**Quadruple Wild-type (qWT) GIST**

SDHB+  
IGF1R-  
Any age?  
Equal sex?  
Site?  
Multifocality?

- Genomics?  
- Epidemiology?  
- Disease Biology?

Lack Mutations in *KIT*, *PDGFRA*, RAS Pathway (*NF1*, *RAS*, *BRAF*) and SDH Subunits

**Quadruple Wild-type (qWT) GIST**

SDHB+ IGF1R-
Any age? Equal sex? Site? Multifocality?

FGFR1 fusions
ETV6-NTRK3 fusions
ARID1B, ATR, LTK, SUFU, ZN217, PARK2 mutations
30s-50s M≥F
Stomach > SB > Colon
Nodal and Distant Mets

NIH Wild-Type GIST Clinic: *KIT-PDGFRA* fusion

## 9 Known Gene Fusions in GIST

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (Years)</th>
<th>Sex</th>
<th>Primary Tumor Location</th>
<th>Tumor Stage</th>
<th>Gene Fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>55</td>
<td>Male</td>
<td>Small bowel</td>
<td>T3N0M1</td>
<td>ETV6-NTRK3</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>Male</td>
<td>Colon</td>
<td>Unknown</td>
<td>ETV6-NTRK3</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>Male</td>
<td>Rectum</td>
<td>T2NxM0</td>
<td>ETV6-NTRK3</td>
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<tr>
<td>4</td>
<td>54</td>
<td>Male</td>
<td>Pelvis mass</td>
<td>Unknown</td>
<td>FGFR1-TACC1</td>
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<tr>
<td>5</td>
<td>54</td>
<td>Male</td>
<td>Stomach</td>
<td>T3N1M1</td>
<td>FGFR1–TACC1</td>
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<tr>
<td>6</td>
<td>38</td>
<td>Female</td>
<td>Small bowel</td>
<td>T3N1M1</td>
<td>FGFR1–HOOK3</td>
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<tr>
<td>7</td>
<td>Unknown</td>
<td>Female</td>
<td>Unknown</td>
<td>Unknown</td>
<td>KIT-PDGFRA</td>
</tr>
</tbody>
</table>
| 8       | 63          | Female| Small bowel      | T3N0M1       | MARK2-PPFIA1
|         |             |       |                       |              | SPRED2-NELFCD       |
| 9 *     | 30          | Female| Small bowel      | T4NxM0       | PRKAR1B-BRAF        |

### Summary

| Average: 48 | Median: 54 | Male 56% | Female 44% | 44% small bowel, but spans stomach to rectum | 22% nodal metastases | 44% distant metastases | 33% ETV6-NTRK3 | 33% FGFR1 | 33% Others |

* UCSD Patient (unreported to date)
Progressive Fragmentation of “WT” GIST

Nannini et al., JTM. May 2017.
Abandoning WT GIST

The Call of “The Wild”-Type GIST: It’s Time for Domestication

Maha Alkhuziem, MBBS, MAS; Adam M. Burgoyne, MD, PhD; Paul T. Fanta, MD; Chih-Min Tang, PhD; and Jason K. Sicklick, MD

Alkhuziem et al., JNCCN. May 2017.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Pathways/Signaling</th>
<th>Matching FDA-Approved, On-Label Agents With Targets in GIST</th>
<th>Matching FDA-Approved, Off-Label Agents With Targets in GIST</th>
<th>Clinical Trials Enrolling Patients With GIST</th>
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</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>MAPK</td>
<td>MEK inhibitors: cobimetinib, trametinib</td>
<td>TKI: crizotinib</td>
<td></td>
</tr>
<tr>
<td>LTK</td>
<td>Transcriptional regulation Insulin receptor signaling</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF1</td>
<td>MAPK</td>
<td>MEK inhibitors: cobimetinib, trametinib</td>
<td></td>
<td></td>
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<tr>
<td>NRAS</td>
<td>MAPK</td>
<td>MEK inhibitors: cobimetinib, trametinib</td>
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<td></td>
</tr>
<tr>
<td>PARK2</td>
<td>E3 ubiquitin ligase Cyclin-CDK complexes</td>
<td>CDK4/6 inhibitor: palbociclib</td>
<td></td>
<td>Phase II (CDK4/6 inhibitor): palbociclib</td>
</tr>
<tr>
<td>PDGFRA</td>
<td>MAPK P13K/AKT/mTOR JAK/STAT</td>
<td>Imatinib (first line) Sunitinib (second line) Regorafenib (third line)</td>
<td>TKI: ponatinib</td>
<td>Phase I (PDGFRA/TKI inhibitors): BLU-285, DCC-2618 Phase II (PDGFRA/TKI inhibitors): dovitinib, famitinib, olaratumab, onalespib, motesanib Phase III (PDGFRA inhibitor): crenolanib</td>
</tr>
<tr>
<td>SDHA</td>
<td>Epigenetic methylation HIF1-alpha expression</td>
<td>Hypomethylating agents: 5-azacytidine, decitabine</td>
<td></td>
<td>Phase I (glutaminase inhibitor): CB-839</td>
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<tr>
<td>SDHB</td>
<td>Epigenetic methylation HIF1-alpha expression</td>
<td>Hypomethylating agents: 5-azacytidine, decitabine</td>
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<td>Phase I (glutaminase inhibitor): CB-839</td>
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<tr>
<td>SDHC</td>
<td>Epigenetic methylation HIF1-alpha expression</td>
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<td>Phase I (glutaminase inhibitor): CB-839</td>
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<tr>
<td>SDHD</td>
<td>Epigenetic methylation HIF1-alpha expression</td>
<td>Hypomethylating agents: 5-azacytidine, decitabine</td>
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<td>Phase I (glutaminase inhibitor): CB-839</td>
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<tr>
<td>SUFU</td>
<td>Hedgehog pathway</td>
<td>GLI inhibitor: arsenic trioxide</td>
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<td></td>
</tr>
<tr>
<td>ZNF217</td>
<td>Transcriptional regulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>Pathways/Signaling</td>
<td>Matching FDA-Approved, On-Label Agents With Targets in GIST</td>
<td>Matching FDA-Approved, Off-Label Agents With Targets in GIST</td>
<td>Clinical Trials Enrolling Patients With GIST</td>
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<tr>
<td>----------</td>
<td>--------------------</td>
<td>-----------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>--------------------------------------------</td>
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<tr>
<td>ARID1A</td>
<td>Chromatin remodeling</td>
<td>mTOR inhibitors: everolimus, temsirolimus</td>
<td>Phase I (PI3K inhibitors): alpelisib, buparlisib, TGR-1202</td>
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<tr>
<td>ARID1B</td>
<td>Chromatin remodeling</td>
<td>mTOR inhibitors: everolimus, temsirolimus</td>
<td>Phase I (PI3K inhibitors): alpelisib, buparlisib, TGR-1202</td>
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<td>ATR</td>
<td>DNA repair</td>
<td>DNA damaging agents: cisplatin, gemcitabine, topotecan, PARP inhibitors: olaparib, rucaparib, Radiotherapy</td>
<td>Phase I (PI3K inhibitors): alpelisib, buparlisib, TGR-1202</td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>MAPK</td>
<td>Regorafenib (third line)</td>
<td>BRAF V600E inhibitors: dabrafenib, vemurafenib</td>
<td>Phase II (BRAF V600E inhibitor): dabrafenib</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MEK inhibitors: cobicetinib, trametinib</td>
<td>Phase II (MEK inhibitors): binimetinib, trametinib</td>
</tr>
<tr>
<td>ETV6-NTRK3</td>
<td>MAPK</td>
<td>TKI: crizotinib</td>
<td>Phase I (TRK inhibitor): larotrectinib</td>
<td>Phase II (TRK inhibitor): entrectinib</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FGFR1</td>
<td>FGF</td>
<td>Regorafenib (third line)</td>
<td>FGFR inhibitors: lenvatinib, pazopanib, ponatinib</td>
<td>Phase I (FGFR inhibitors): BGJ398, dovitinib</td>
</tr>
<tr>
<td>HRAS</td>
<td>MAPK</td>
<td>MEK inhibitors: cobicetinib, trametinib</td>
<td>Phase I (MEK inhibitors): cobicetinib, trametinib</td>
<td>Phase II (FGFR inhibitor): semaxanib</td>
</tr>
<tr>
<td>KIT</td>
<td>MAPK</td>
<td>Imatinib (first line)</td>
<td>TKIs: dasatinib, nilotinib, ponatinib</td>
<td>Phase I (TKIs): DCC-2618, OSI-930, PLX9486</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sunitinib (second line)</td>
<td></td>
<td>Phase II (TKIs): BBi503, cabozantinib, dasatinib, farnitinib, ganetespib, nilotinib, pexidartinib, sorafenib, sunitinib, Phase III (TKI): masitinib</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regorafenib (third line)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary #1

- "Quadruple Wild-Type: or “Unclassified” GIST occur in younger patients, occur in similar locations as non-qWT GIST, frequently metastasize to lymph nodes, and most are not truly “WT.”

- Potentially deleterious gene fusions occur in adults with GIST and these are potentially targetable with drugs.
  - KIT inhibitors (KIT-PDGFRA fusion)
  - NTRK3 inhibitors (ETV6-NTRK3 fusion)
  - FGFR1 inhibitors (FGFR1-TACC1/HOOK3 fusions)
  - BRAF inhibitors (BRAF-PRKAR1B fusion)

- Other driver genes at play:
  - ARID1A/D, ATR, LTK, MAX, PARK2, SUFU, ZNF217

Alkhuzeim et al., JNCCN. May 2017.
Is Location is a Biomarker for Gene Mutations?

Location. Location. Location. Location.

Why WHERE you buy is more important than WHAT you buy.
Known Driver Genes in GIST

Pantaleo et al., Cancer Medicine. 2015.
Anatomic Localization of GIST

- **Stomach**: 55%
- **Colon**: 3%
- **Small Intestine**: 29%
  - Duodenum
  - Jejunum
  - Ileum

Duodenal-Jejunal Flexure (DJF) [Ligament of Treitz]?
Genes and Localization of GIST

- **Stomach**: 55%
- **Colon**: 3%
- **Small Intestine**: 29%

**Germline NF1**

**KIT Exon 9**

**PDGFRA & SDH**

- **Duodenum**
- **Jejunum**
- **Ileum**

**Duodenal-Jejunal Flexure (DJF)**

*Ma et al., CEBP. 2015.*
1. Often multifocal small intestine GISTs associated with Neurofibromatosis type 1 (NF-1)
2. NF-1 associated with 1.5% of GISTs
3. Somatic *NF1* mutant small bowel GIST was recently reported in the absence of a germline *NF1* mutation (Belinsky *et al.*, *BMC Cancer*, 2015).
4. *NF1* gene mutations associated with NF-1 were recently reported (Gasparotto *et al.*, *Clin Cancer Research*, 2016):
   - Frequent in GISTs lacking *KIT/PDGFRA/BRAF* mutations or *SDH* inactivation
   - Especially if multifocal or with a multinodular growth pattern and a non-gastric location.
New Key Findings

1. In three series, GISTs more frequently than 1.5% possess *NF1* genomic alterations
   - 6.1% (MSKCC, 7/115)
   - 9.7% (UCSD, 6/62)
   - 9.7% (FMI, 18/186)
New Key Findings

Germline NF1 KIT Exon 9

- PDGFRA & SDH
- Stomach
- Duodenal-Jejunal Flexure (DJF)
  - 5.5% of GISTs
  - Germline NF1 mutations even without clinical NF-1
  - Somatic only NF1 mutations
  - Can have NF1 + KIT mutations

Small Intestine
- Duodenum
- Jejunum
- Ileum

Colon
Methods

Primary Study Population
• Retrospective study of 165 GIST patients with from January 1, 2000 to April 30, 2017 at the UC San Diego Moores Cancer Center
• Data collected included age, sex, race, ethnicity, primary GIST site, tumor size, and mitotic index.

Next Generation Sequencing
• 62 patients underwent NGS of cancer-related genes beginning in 2014:
  • Foundation Medicine (315 genes)
  • UC San Diego Heath System Clinical Genomics Laboratory (397 genes)

Burgoyne et al., JCO Precision Oncology. 2017.
Driver Mutations in 62 UCSD GIST

- **KIT 63%**
- **PDGFRα 11%**
- **SDH [ABCD] 11%**
- **NF1 9%**
- **BRAF 3%**
- **KRAS 3%**
**NF1 Genomic Alterations are Frequent at DJF**

- **GIST Patients**
  - NGS: N = 62 (37.6%)
  - No NGS: N = 103 (62.4%)

- **NF1 Alteration**
  - N = 6 (9.7%)

- **No NF1 Alteration**
  - N = 56 (90.3%)

- **Stomach**
  - N = 1 (16.7%)
  - DJF: N = 5 (83.3%)

- **No NGS**
  - DJF: N = 2 (2.1%)
### 9 DJF GIST Patient Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>55 (36-80)</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>55.9 ± 15</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>44.4%</td>
</tr>
<tr>
<td>Female</td>
<td>5</td>
<td>55.6%</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
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<tr>
<td>Caucasian</td>
<td>7</td>
<td>77.8%</td>
</tr>
<tr>
<td>African American</td>
<td>1</td>
<td>11.1%</td>
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<tr>
<td>Asian/Pacific Islander</td>
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<td>11.1%</td>
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<tr>
<td><strong>Ethnicity</strong></td>
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<tr>
<td>Non-Hispanic white</td>
<td>5</td>
<td>55.6%</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>4</td>
<td>44.4%</td>
</tr>
</tbody>
</table>

Burgoyne et al., JCO Precision Oncology. 2017.
### DJF GIST Clinicopathologic Features

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>%</th>
</tr>
</thead>
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<td><strong>Stage</strong></td>
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<tr>
<td>Localized</td>
<td>6</td>
<td>66.7%</td>
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<tr>
<td>Regional</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Distant</td>
<td>1</td>
<td>11.1%</td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>22.2%</td>
</tr>
<tr>
<td><strong>Tumor Size, cm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>9 (1.5 - 15)</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>8.0 ± 5.0</td>
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</tr>
<tr>
<td><strong>Mitotic Index</strong></td>
<td></td>
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</tr>
<tr>
<td>Low</td>
<td>4</td>
<td>44.4%</td>
</tr>
<tr>
<td>High</td>
<td>3</td>
<td>33.3%</td>
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<tr>
<td>Unknown</td>
<td>2</td>
<td>22.2%</td>
</tr>
<tr>
<td><strong>Cell Morphology</strong></td>
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<tr>
<td>Spindle</td>
<td>5</td>
<td>55.6%</td>
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<tr>
<td>Epithelioid</td>
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<td>0.0%</td>
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<tr>
<td>Mixed</td>
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<td>33.3%</td>
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<tr>
<td>Unknown</td>
<td>1</td>
<td>11.1%</td>
</tr>
</tbody>
</table>

Burgoyne et al., JCO Precision Oncology. 2017.
**MSKCC Validation Cohort**

- **MSK-IMPACT NGS** (341 genes)  
  N = 115

- **NF1 Alteration**  
  N = 7  
  (6.1%)

- **Multifocal**  
  N = 2  
  (28.6%)

- **Unifocal**  
  N = 5  
  (71.4%)

- **DJF (No Clinical NF-1)**  
  N = 2  
  (40%)

---

Burgoyne et al., *JCO Precision Oncology*. 2017.
<table>
<thead>
<tr>
<th>CASE</th>
<th>Size (cm)</th>
<th>Tumor</th>
<th>Mitotic Index</th>
<th>Genomic Alteration</th>
<th>Reported GIST Drivers</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>MI (per 5 mm²)</td>
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<td></td>
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<tr>
<td>1</td>
<td>15</td>
<td>3</td>
<td>High</td>
<td>Nonsense</td>
<td>CDC73</td>
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<tr>
<td>2</td>
<td>11</td>
<td>1.5</td>
<td>Low</td>
<td>Frameshift</td>
<td>EP300</td>
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<tr>
<td>3</td>
<td>2</td>
<td>13</td>
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<td>Missense</td>
<td>NOTCH2</td>
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<tr>
<td>4</td>
<td>1</td>
<td>5.3</td>
<td></td>
<td>In frame indel</td>
<td>MAML2</td>
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<tr>
<td>5</td>
<td>10</td>
<td>3</td>
<td></td>
<td>Deletion</td>
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<tr>
<td>6</td>
<td>7</td>
<td>1</td>
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<td>Splicing</td>
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<td>7</td>
<td>6</td>
<td>2.5</td>
<td></td>
<td>Notch Pathway</td>
<td></td>
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<tr>
<td>8</td>
<td></td>
<td>2.1</td>
<td></td>
<td>Others</td>
<td></td>
</tr>
</tbody>
</table>

Burgoyne et al., JCO Precision Oncology. 2017.
Summary #2

• Duodenal-Jejunal Flexure (DJF) or Ligament of Treitz GISTs frequently possess NF1 alterations (somatic and/or germline), which occur even in the absence of clinical NF-1.

• This represents a previously unappreciated presentation of clinical NF-1.

Solitary GIST arising at the DJF may be a biomarker for clinically occult NF-1, even if single gene testing reveals a KIT mutation.
NF1 and Notch Genomic Alterations

Lobry et al., Blood. 2014.

Dumont et al., Carcinogenesis. 2012.
Clinical Implications

Any DJF GIST may be considered for \textit{NF1} gene analysis, and any positive result has the following clinical implications:

1. Additional cancer screening according to expert guidelines.
2. Familial genetic counseling and screening.
3. Personalizing systemic therapy as \textit{NF1} mutant GISTs tend to be imatinib-resistant.

Burgoyne et al., JCO Precision Oncology. 2017.
Slicing the Pie...It’s Time for Personalization

Shi et al., JTM. 2016.
Funding

UC San Diego

NIH

SSAT

PEDAL THE CAUSE
A WORLD WITHOUT CANCER

The Kristen Ann Carr Fund

SDH-RA
SDH-Deficient GIST Research Advocates

Hope for a Cure Foundation
Support and Hope in the Quest for a Cure

Anonymous Patient-Advocate Donors
(UCSD Foundation Fund #3661)

GIST Support International

The Life Raft Group

UC San Diego Moores Cancer Center
Acknowledgements

Sicklick Lab
Chih-Min Tang
Adam Burgoyne
Eileen Shi
Mayra Yerba
Sudeep Banerjee
Grace Ma
Taylor Coe
Kelly Fero
Tracy Lee

UCSD
Paul Fanta
Adam Burgoyne
Olivier Harismendy
John Thorson
James Murphy
Lisa Madlensky
Elena Martinez
Dwayne Stupack
Michael McHale

U of Miami
Jon Trent

MDACC
Neeta Somaiah
David Hong
Billy Wang

MSKCC / U Penn
Ron DeMatteo

OHSU
Michael Heinrich
Chris Corless

Mayo Clinic
Tamas Ordog
Sabriya Syed

Columbia
Gary Schwartz
Andrea Califano