GIST 101: The Biology of GIST (and a little bit of the medicine, too)

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Disclaimer: I am not a physician. I am a scientist (biochemistry/toxicology) with some experience in cancer research.

Nothing in this presentation should be regarded as medical advice or as a substitute for consulting with your doctors.
TOPICS

- What causes GIST?
- Where do GISTs come from (cell types)?
- What is “KIT”?

*Crash course in Molecular Biology!*

- How do “TKI” drugs (such as Gleevec) work?
- The “new generation” of GIST drugs
What causes GIST?

Most GISTs (>97%) occur “sporadically” (randomly); neither inherited nor passed on within families.

\textit{GIST strikes at random.}

No environmental, geographical, occupational, dietary, or lifestyle causes of sporadic GIST are known - and if there were any major risk factors, they would have been noticed by now!
Several *rare* (3%?) familial forms of GIST are known.

- *NF1* (Neurofibromatosis)
- Germline *KIT* mutations
- SDH-deficient GIST


*This presentation is limited to sporadic GIST.*
• What causes GIST?

• Where do GISTs come from (cell types)?

• What is “KIT”?

• How do “TKI” drugs (such as Gleevec) work?

• The “new generation” of GIST drugs
There are hundreds of different types of cells in the body.

- cardiomyocytes (heart)
- hepatocytes (liver)
- white blood cells
- adipocytes (fat tissue)

The cancers that arise from these cells are just as different!
Cancers can begin in almost any type of cell in the body.

The type of cell from which it develops defines the biology of the cancer - and determines its treatment.

The pathologist is tasked with identifying the cell type (usually, by studying a biopsy/surgery specimen).

The medical oncologist uses that information to plan the course of treatment.
It is the **cell type** - not the **organ** - that defines a cancer.

*Basal cell carcinoma* and *malignant melanoma* are both “skin cancers” but they are completely different diseases.

*Adenocarcinoma* and *mesothelioma* are both “lung cancers” but they are completely different diseases.
Carcinomas vs. sarcomas: different classes of cancers

Carcinomas - the most common cancers - skin, colon, lung, prostate, breast, etc. - arise in epithelial (“lining”) cells.

GIST is not a carcinoma; it is a sarcoma - a cancer that arises from cells of the connective tissues - muscle, cartilage, bone, etc.

Sarcomas are rare (about 1% of human cancers).

Treating sarcomas is a sub-specialty among oncologists; treating GISTs is a sub-sub-specialty!
The Gastrointestinal Tract: a 5-metre-long tube.

GI carcinomas are common (stomach cancer, colon cancer, etc.)

GIST (sarcoma) is rare.
Cross-section of the GI tract; the interior (lumen) of the tract is (topologically) *outside* the body.
Cross-section of the GI tract

carcinomas start in the epithelial lining (the body’s “outside” surface)

GISTs (sarcomas) start in the muscular wall
Interstitial Cells of Cajal: the cells where GISTs start; the “pacemaker” cells that coordinate GI peristalsis.

**Peristalsis** - the coordinated waves of muscle action that push food through the GI tract during digestion.

The coxswain keeps the crew’s muscles synchronized.
Interstitial cells of Cajal are the “pacemaker” cells that coordinate GI tract peristalsis. ICCs send out the electrical pulses that stimulate the waves of contraction of the muscle surrounding the GI tract.

ICCs are the cells where GISTs start.

ICCs: pacemaker electrical activity (mouse)

The coxswain keeps the crew’s muscles synchronized.
GIST tumors arise in the same cell type (ICC), regardless of their location along the GI tract.
A GIST that starts in the stomach is a GIST

(... not what people are usually referring to when they say “stomach cancer” - the common adenocarcinoma).

A GIST that starts in the colon is a GIST

(... not what people are usually referring to when they say “colon cancer” - the common colorectal carcinoma).
GISTs, like other cancers, can metastasize - spread from the “primary” tumour to new sites in the body. GISTs tend to spread to the liver and the peritoneum (the membrane lining the abdominal cavity).
At the time of diagnosis, a GIST may still be localized, or it may already have become metastatic.

A localized GIST *may be cured* by surgery; but, even after successful surgery, GIST may recur.

If the GIST has metastasized, it *cannot* be cured by surgery alone (although surgery may be performed).

Systemic (drug) therapy is needed.
Metastasis:

At the time of diagnosis, a GIST may be localized or it may have spread (metastasized), e.g., to the liver or lung.

GIST metastases are still GISTs and must be treated as GISTs

... they are not “liver cancer” or “lung cancer”.

STING Englishman in NEW YORK

An Englishman in New York is still an Englishman.
• What causes GIST?
• Where do GISTs come from (cell types)?
• What is “KIT”? 

• How do “TKI” drugs (such as Gleevec) work?
• The “new generation” of GIST drugs
The Molecular Biology of GIST ...

and a crash course in Molecular Biology!

- GIST cells almost always express a protein called “KIT” (very few other cells in the body do so)
- In most cases of GIST, the \textit{KIT} gene is mutated, producing an aberrant form of KIT protein that “drives” cell division and therefore drives the cancer.

Yukihiro Kitamura, M.D.         Seiichi Hirota, M.D.
Osaka Univ. Med. School
The GIST-KIT connection (2020 update)

We now realize that “GIST” is an “umbrella” term that encompasses several sarcomas, differing at the molecular level.

Most GISTs are “KIT-mutant”, but about 25% are not: they carry (and express) the “wild-type” (normal) form of KIT.

- About 15% have a mutation in a related gene, PDGFR.
- A few have mutations in another gene, e.g. RAS, BRAF, NF1, NTRK, or SDH .... and probably a few others, still unknown.

These less-common forms of GIST are distinct from KIT-mutant GIST, in terms of their biology and treatment.

Note: All of these forms of GIST are derived from ICCs and they all* express KIT protein - whether or not the KIT gene is mutated.

*almost all, anyway; there are very rare exceptions.
**KIT ("c-Kit" or "CD117")**

KIT protein is made (expressed) by only a few types of adult cells, including ICCs (and GISTs).

*Immunohistochemistry (IHC):*

The essential step in diagnosing GIST is to test whether the tumor cells express KIT protein.

The test is performed by *staining* the tissue sample (obtained at surgery) with an *antibody* that recognizes KIT protein. The stained tissue is examined under the microscope.

If the cells stain brown, they are almost certainly GIST.

Di Vizio *et al.*, 2008
IHC: The tumour expresses KIT (and DOG1), so it is almost certainly GIST.

Consistent with this evidence, the tumour does not express proteins (e.g., desmin) that are usually expressed by certain sarcomas other than GIST.

IHC does not distinguish between normal (“wild-type”) and mutated forms of KIT.
**Immunohistochemistry (IHC) vs. Mutational testing: Different tests, different questions, different answers**

<table>
<thead>
<tr>
<th></th>
<th>Immunohistochemistry (staining for KIT protein)</th>
<th>Mutational testing (DNA sequencing of <em>KIT</em> gene)</th>
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<tbody>
<tr>
<td>Tests for:</td>
<td>expression of KIT protein by the tumour cells</td>
<td>mutations in the <em>KIT</em> gene in the tumour cell DNA</td>
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<tr>
<td>Tells us:</td>
<td>whether the tumour is a GIST (often, merely confirming the diagnosis)</td>
<td>whether the tumour is a KIT-mutant GIST (and, if so, identifies the mutation)*</td>
</tr>
<tr>
<td>Requires:</td>
<td>tumour sample (biopsy or surgery)</td>
<td>tumour sample (<em>e.g.</em>, FFPE: Formalin-Fixed Paraffin-Embedded)</td>
</tr>
<tr>
<td>Performed by pathology lab?</td>
<td>always</td>
<td>sometimes; LRG strongly recommends that patients push to have mut. testing done!</td>
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</table>

*If no mutation is seen in the *KIT* gene, the lab will probably go on to look at other genes, *e.g.* PDGFR, RAS, BRAF ...
Mutational testing identifies a \textit{PDGFR} mutant GIST.

\begin{verbatim}
\textbf{RESULTS}
NGS Panel: Actionable variant(s) detected

\textbf{Variant 1}
Gene: PDGFR
Variant: c.2525A>T (p.Asp842Val)
Variant allele frequency %: 45

Note: If “no variants detected” is reported, this indicates that no clinically relevant variants were detected in the genes listed below.

\textbf{CLASS 1 Variants: Variants actionable in the disease site in which they have been identified.}

\textbf{PDGFR} (NM_006206.4) c.2525A>T (p.Asp842Val)

The \textbf{PDGFR} gene is recurrently mutated in GIST (mycancergenome.org). The p.Asp842Val variant in \textbf{PDGFR} is associated with resistance to treatment with tyrosine kinase inhibitors imatinib and sunitinib (mycancergenome.org; PMID: 15028335). Preclinical studies suggest that this variant is responsive to dasatinib, but not sorafenib or nilotinib (PMID: 18794084).

\textbf{_genes Tested:}

Melanoma: BRAF [NM_004333.4], NRAS [NM_002524.3], KIT [NM_000222.2], GNAQ [NM_002072.4], GNA11 [NM_002067.4]

Colorectal cancer: BRAF [NM_004333.4], KRAS [NM_003330.3], NRAS [NM_002524.3], PIK3CA [NM_008218.2]

Lung cancer: AKT1 [NM_00101432.1], BRAF [NM_004333.4], EGFR [NM_005228.3], ERBB2 [NM_004448.3], KRAS [NM_003330.3], PIK3CA [NM_008218.2], RET [NM_020975.4], TP53 [NM_000546.5]

\textbf{GIST: KIT [NM_000222.2], PDGFR [NM_006206.4]}

\textbf{Methodology:} DNA was extracted from the paraffin-embedded soft tissue (tumour GIST), excision, S-18-21327, block A9 and analyzed using the TruSight Tumor 15 Panel (Illumina) on the MiSeq next-generation sequencing platform (Illumina). Data generated were analyzed for genes as listed above.

The lower limit of detection: 3-10\% mutant allele frequency.
\end{verbatim}
Genes and Proteins

Genes (DNA) are the codes ("construction blueprints") for the cell’s proteins. The human genome encodes >30,000 different kinds of proteins.

Different cells make different sets of proteins.

The genome is the “library”; different cells “read different books”:

- Muscle cells make actin and myosin (etc.)
- Red blood cells make hemoglobin (etc.)
- Neurons make ion channels (etc.)
- etc. etc. etc. etc. etc. etc.
Genes and Proteins

Genes (DNA) are the codes ("construction blueprints") for the cell’s proteins. The human genome encodes >30,000 different kinds of proteins.
Proteins

Proteins are linear sequences of building blocks: amino acids, of which there are 20:

- A = alanine
- C = cysteine
- D = aspartic acid
- E = glutamic acid
- F = phenylalanine
- H = histidine
- K = lysine
  
extc.

A protein sequence may be anywhere from about 100 to many thousands of amino acid residues in length.
The KIT protein: 976 amino acid residues

1  MRGARGAWDF LCVL LLLLRV QTGSSQPSVS PGEPSPPSIH PGKSDLIVRV GDEIRLLCTD
61  PGFVKWTFEI LDETNENKQN EWITEKA EAT NTGKYTCTNK HGLSNSIYVF VRDPAKLFLV
121  DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKKLPKDL RFI PDPKAGI MIKSVKRAYH
181  RLC LHC SVDQ EGKSVLSEKF ILKVRPAFK A VPVVSVSKAS YLLREGEEFT VTCTIKDVSS
241  SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN
301  VTTTLEVV DK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQW IY MNRTFTDKWE
361  DYPKSENESN IRYVSELHLT RLG TEGGTY TFLVSNSDVN AAIAFNVYVN TKPEILTYDR
421  LVNGMLQCVA AGFPEPTIDW YFC PGT EQRC SASVLPVVDQ T LNSSGPPPFG KL VQSSIDS
481  SAFKHNGTV E CKAYNDV GKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMCLI IV
541  MILTYKYLQK PMYE VQWKV V EEINGNNYVV IDPTQLPYDH KW EFPRNRLS FG KTLGAGAF
601  GKVVEATAYG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LK VLSYLGNH MNIVNLLGAC
661  TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSQ EDHA EA A LYKN LHSK ESSCS DSTNE
721  YMDMKPGVSY VVPTKADKRR S V RIGSYIER DVTPAIME D EL AL DLED LL SFSYQVAKGM
781  AFLASKNCI H RDLAARNILL THGTRITKICD FG LARDIKND SNYVVKGNAR LPV KW MAPES
841  IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSK FY KMIKEGFRML SPEHAPAEMY
901  DIMKTCWDAD PLKRPTFKQI VQ LIEK QISE STNHIYSNLA NCSPNRQKPV VDHSV RINSV
961  GSTASSSQPL LVHDDV
Protein structure: Exons*

Proteins consist of multiple distinct “domains” (sub-structures), each 30-100 amino acids:

Each domain corresponds to a separate segment of the gene coding for that protein; these gene segments are called exons. The *KIT* gene has 21 exons.

Genome: Library
Protein: Book
Exon/Domain: Chapter
Amino acid: Letter

*This is an over-simplified discussion of exons and domains*
The KIT protein: 976 amino acid residues

1  MRGARGAWDF  LCVLLLLLRV  QTGSSQPSVS  PGEPSPPSIH  PGKSDLIVRV  GDEIRLLCTD
61  PGFVKWTFEI  LDETNENKQN  EWITEKAEEAT  NTGKYTCTNKL  HGLSNSIYVF  VRPDPAKLFLV
121  DRSLYGKEDN  DTLVRCPLTD  PEVTNYSLKGL  CQGKPLPKDPL  RFIKPDPKAGIMIKSVKRAYH
181  RLCLHCSVDQ  EGKSVLSEKEF  ILKVPRPAFKA  VPVVSVSKAS  YLLREGEEFTVTCTIKDVSS
241  SVYSTWKREN  SQTKLQEKYNSW  WHGDFNYESQ  ATLTISSARVNDGVFMCYANNTFGSAN
301  VTTTLLEVVDK  GFINIFPMIN  TTVFVNDGEN  VDLIVEYEAFPKPEHQQWYIMNRTFTDKWE
361  DYPKSENESN  IRYVSELIHLT  RLKGTTEGTYTF  LVSNSDVNAIAAFNYVNTKPEILTYDR
421  LVNGMLQCVA  AGFPEPTIDWYFCPGTEQRC  SASVLVVDQ  TLRSSSGPPFGKLVQSSIDS
481  SAFKHNGTVE  CKAYNDVGTKTSAYFNFAFKG  NNKEQIHPHTLFTPPLLIGFIVAGMMCIIV
541  MILTYKLYQK  PMYEVTQKVVEEINGNYVYIDPTQLP YDHKWEFPRNRLSFGKTLGAGAF
601  GKVVEATAYG  LIKSDAAMTVAVKMLKPSAHLTEREALMSELKVLSLGNHMNIVNLLGAC
661  TIGGPTLVIT  EYCCYGDLLNFLRRKRDSTISCSKQEDHAEAALYKNLLHSESSCSDSTNE
721  YMDMKGVSYYV  VVPTKADKRRSVRIGSYIERDIVTPAMEDD  ELALDLEDLLSF SYQVAKGM
781  AFLASKNCIH  RDLAARNILLTHGRITKICDFGLARDIKNDSNYVVKGNARLPVKWMAPES
841  IFNCVYTFES  DVWSYGIFLWELFSLGSSPYPGMPVDSKFYKMIKEGRFMLSPEHAPAEMY
901  DIMKTCWDAD  PLKRPTFKQIQVQLEKQISESTNHIYSNLANCSPNRQKPVDHHSVRSINV
961  GSTASSSQPL  LVHDDV
The KIT protein: 21 exons

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121  DRSLYGKEDN  DTLVRCPLTD  PEVTNYSLKG  CQGKPLPKDL  RFIPDPKAGI  MIKSVKRAYH
181  RLCLHCSDVQ  EGKSVLSEKF  ILKVRPAFKA  VPVVSVSKAS  YLLREGEEFT  VTCTIKDVSS
241  SVYSTWKREN  SQTKLQEKYN  SWHHGDFNYE  RQATLTISSA  RVNSGAVFMC  YANNTFGSAN
301  VTTTLEVVDK  GFNIFPMIN  TTVFYNDGEN  VDLIVEYEAF  PKPEHQQWYI  MNRTFTDKWE
361  DYPKSENESN  IRYVSELHLT  RLMGTEGGTY  TFLVSNSDYN  AAIAFNVYVN  TKPEILTYDR
421  LVNGMLQCVA  AGFPEPTIDW  YFCPGTEQRC  SASVLPVDVQ  TLNSSGPFFG  KLVQSSIDS
481  SAFKHNQGVT  ECKAYNDVGKT  SAYFNFAFKG  NNNKEQIHPT  LFTPLLIGFV  IVAGMMCIIV
541  MILTYKYLQK  PMYEVQWKVY  EEINGNYYVY  IDPTQLPYDH  KWEFPRNRLS  FGKTLGAGAF
601  GKVVEATAYG  LIKSDAAMTV  AVKMLKPSAH  LTERAEMLSE  LKVLSYNLNH  MNIVNLGAC
661  TIGGPTLVIT  EYCCYGDLLN  FLRRKRDSFI  CSQEDHAAE  ALYKNLHSK  ESSCDSTNE
721  YMDMKGPSY  VVPTKAADKR  SVRIQSYIER  DVTPAIMEDD  ELALDLEDLL  SFISHQVAKGM
781  AFLASKNCIH  RDLAARNILL  THGRITKICD  FGLARDIKND  SNYVVGNAR  LPVKWMAPES
841  IFNCVYTFES  DVWYSIGIFLW  ELFSLGSSPY  PGMPVDSKGY  KMIKEGRML  SPEHAPAEYM
901  DIMKTCWDAD  PLKRPTFKQI  VQLIEKQISE  STNHAYSNLAR  NCSPNRQKPV  VDHESRINSV
961  GSTASSSQPL  LVHDDV
The KIT protein: 21 exons

<table>
<thead>
<tr>
<th>Exon</th>
<th>Amino Acid Sequence</th>
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<tr>
<td>1</td>
<td>MRRARGAVDF LCVLLLLLRRV QTGSSQPSVS PGEPPPSIHI PGKSDLIVRV GDEIRLLCTD</td>
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<tr>
<td>61</td>
<td>PGFVKWTFEI LDETNENKQNEWITEKAEAT NTGKYTCTFKNGHLSNSIYVF VRTDPAKLFLV</td>
</tr>
<tr>
<td>121</td>
<td>DRSLYGKEDN DTLVRCPPLTD PEVTNSYSLKG CQGKPLPKDL RFIPDPKAGIMIKSVKRAYH</td>
</tr>
<tr>
<td>181</td>
<td>RLCLHCSDVDQ EGKSVLSEKF ILKVRPAFKA VPVSVSOKZS YLLREGEFFT VTCTIKDVSS</td>
</tr>
<tr>
<td>241</td>
<td>SVYSTWKRENSQTKLQEKYN SWHHGDFNYE RQATLITISSA RVDNSGVFMC YANNTFGSAN</td>
</tr>
<tr>
<td>301</td>
<td>VTTTLEVVDK GFIFIFPMIN TTVFVNDEG VDLIVEYEADF PKPEHQQQWIYMNRTFTDKWE</td>
</tr>
<tr>
<td>361</td>
<td>DYPKSENESN IRYVSELHLLT RLKGTEGGTY TFLVSNDSVNNAAIAFNYVNYNTKPEILTYDR</td>
</tr>
<tr>
<td>421</td>
<td>LVNGMLQCVA AGFPEPTIDWR YFCPGTEQRC SASVLPVDVQ TLNSGPPFGKLVVQSSIDS</td>
</tr>
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<td>481</td>
<td>SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHHPHT LFTPLLIGFVIVAGMMCIIV</td>
</tr>
<tr>
<td>541</td>
<td>MILTYKLYQKPMYEVQNKVVEEINGNYVYIDPQLPYDHKFEPFRNRLSFGKTLGAGAF</td>
</tr>
<tr>
<td>601</td>
<td>GKVVEATAYAG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNHMINIVNLLGAC</td>
</tr>
<tr>
<td>661</td>
<td>TIGGPTLVIT EYCCYGDLN FLRRKRDSFTCSKQEDHAЕAALYKNLHSEKESSCSFSTNE</td>
</tr>
<tr>
<td>721</td>
<td>YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTAPIMEDD ELALDLEDILLFSFSYQVAKGM</td>
</tr>
<tr>
<td>781</td>
<td>AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKNDSNYVVKGNARLPVKWMAPES</td>
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<tr>
<td>841</td>
<td>IFNCVYTFES DVWSYGIFLW ELFSLSGSPY PGMPVSDKFYSMIKEGFRMLSPEHAPAEMY</td>
</tr>
<tr>
<td>901</td>
<td>DIMKTCWDAD PLKRPTFKQISQVQIEKQISESNTNHIYSNLANCSPNRQKPVVDHISVRINSV</td>
</tr>
<tr>
<td>961</td>
<td>GSTASSSQPL LVHDDV</td>
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</tbody>
</table>
The KIT protein: 21 exons
KIT is one member of a large family of related proteins. PDGFR is a “sister”; ABL is a “distant cousin”.
What is a **mutation**?

- A change in the DNA sequence encoding a protein.

- Mutations occur randomly, but cells carrying certain mutations will die, while others will grow faster.
The KIT protein is an enzyme – a “tyrosine kinase” – that acts on other proteins, modulating their activities (triggering a “signal transduction cascade”).

In about 75% of GIST cases – but not 100% - the KIT gene is mutated; consequently, an aberrant form of KIT protein is produced by the GIST tumour cells.

The KIT gene is an “oncogene”.

An oncogene is a gene which, when mutated, encodes a protein product that can instruct the cell to keep on dividing: a “stuck gas pedal”.

When the KIT gene is mutated, KIT protein acts as a “driver” that tells the GIST cells to proliferate.
KIT mutations in GIST are (almost always) somatic.

The “driver” mutations in GISTs are almost always somatic - not germ-line - mutations.

- occurring in cells of the body during development or adulthood, but not affecting germ cells (egg or sperm cells)

- The somatic KIT mutation is carried by all of the tumor cells, but it cannot be passed on to a patient’s children.
Diversity of mutations in GISTs

GIST “driver” mutations can occur at many different sites in the $KIT$ gene, affecting many different sites in the KIT protein ... and sometimes GIST driver mutations occur in genes other than $KIT$: $PDGFR$, $SDH$, $BRAF$, $NTRK$, etc.

The site of the mutation affects prognosis and response to drugs.

Mutation testing should be performed on all new GIST cases; a sample of the tumour is needed (not just a blood sample).

(Baveno declaration, 2008).
Your specific mutation matters in getting the right treatment for your type of GIST.

Mutations, or abnormal changes in genes, can cause cancer by making cells in the body grow and spread when they are not supposed to. In GIST, mutations lead tumors to develop along with the normal cells of the gastrointestinal tract.

**BRAF**, B-Raf proto-oncogene, serine/threonine kinase; **KIT**, KIT proto-oncogene receptor tyrosine kinase; **KRAS**, K-Ras proto-oncogene, GTPase; **NF1**, neurofibromin 1; **PDGFRA**, platelet-derived growth factor receptor alpha; **SDH**, succinate dehydrogenase.

* SDH deficiency refers to a decrease in succinate dehydrogenase (SDH, heme A-c at high-SDH-deficiency), a protein. The decrease can develop from mutations in specific genes.

In the United States, **3 of 4** people with GIST may not be tested for mutations.

**Mutational testing is the only way to confirm which mutation is causing your GIST.**

Confirming your specific mutation through mutational testing is the best way to ensure that your treatment plan is right for your type of GIST.

**KIT** mutations drive *most* sporadic GISTs.

Approximate distribution of “driver” mutations in GISTs
Exons 9 and 11 are the regions of the KIT gene/protein where most of the primary mutations in GISTs are found.
Exon 11 encodes the “juxtamembrane” domain of the KIT protein.

Exon 11 mutations cause a conformational change ("switch") of KIT protein from its “inactive” to its “active” form, signaling the GIST cell to grow and divide.
Understanding mutation terminology

What does “KIT V560D” mean?

This is a “mis-sense” mutation. Because of a mutation in the GIST cell’s DNA, the 560\textsuperscript{th} amino acid (building block) in the KIT protein has changed from the normal valine (V) to a different residue, aspartic acid (D).

What does “KIT W557_K558 del” mean?

This is a “deletion” mutation. Because of a mutation in the GIST cell’s DNA, the 557\textsuperscript{th} and 558\textsuperscript{th} amino acids in the KIT protein are absent.
KIT-mutant GIST: examples

A section of exon 11 of the normal ("wild-type") KIT protein; each colored block represents a particular amino acid.

After mis-sense mutation V560D ...

After deletion mutation W557_K558 ...
• What causes GIST?
• Where do GISTs come from (cell types)?
• What is “KIT”?

• How do “TKI” drugs (such as Gleevec) work?
• The “new generation” of GIST drugs
The development of targeted drugs for treating GIST

“Targeted” drugs for treating GIST

Chronic Myelogenous Leukemia (CML)

A rare leukemia (cancer of the blood) that looks completely different from GIST ... but the two diseases turned out to be related, at the molecular level.

The mutation causing CML is in a gene called “ABL”; this was discovered in 1985. ABL is a “distant cousin” of KIT.

ABL is a “tyrosine kinase” enzyme. Drugs that inhibit (shut down) those enzymes are “tyrosine kinase inhibitors” (TKIs).
The Royal Swedish Academy of Sciences has decided to award cancer researchers Dennis Slamon and Brian Druker the Sjöberg Prize 2019, worth $1,000,000. The two researchers have been revolutionary in the development of targeted treatments that improve the prognosis for, and survival of, thousands of patients.
Imatinib (gleevec) inhibits ABL
GIST therapy has benefited from CML discoveries.

KIT and PDGFR, like ABL, are tyrosine kinase enzymes.

The “first generation” GIST drugs - imatinib, sunitinib, and regorafenib - were all developed for CML or other cancers, not for GIST - but they work pretty well for GIST, too.

(A “second generation” of “bespoke” GIST drugs is arriving!)
The first three TKIs approved for use in GIST:

First-line: Imatinib (Gleevec - Novartis; 2001)

Second-line: Sunitinib (Sutent - Pfizer; 2006)

Third-line: Regorafenib (Stivarga - Bayer; 2013)

(The ‘ib” ending indicates an enzyme inhibitor)
Imatinib, sunitinib, and regorafenib all act by the same mechanism - blocking the binding of ATP (cellular fuel) to KIT.

“Second generation” GIST drugs, such as ripretinib (to be discussed later), use different mechanisms of KIT inhibition.
Rubin et al., Lancet 2007

KIT-activated signal transduction; GIST proliferation and survival

inhibition of KIT; reduced GIST proliferation; apoptosis (cell death)
Despite the success of these drugs, more are needed:

- Some GISTs are imatinib-resistant from the outset; e.g., the most common PDGFR mutation, D842V.

- Tolerance of the drugs (side effects) is variable.

- Imatinib halts the growth of most GISTs, but does not eliminate them; over time, GIST tumours tend to become imatinib-resistant, mainly due to additional mutations arising in the metastases.
imatinib resistance: secondary mutations in \textit{KIT}

imatinib resistance due to *KIT* secondary mutations: options for switching to other TKIs?

adapted from Serrano and Fletcher, *Oncotarget* 2019
### Differential Sensitivity to TKI

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<th>Primary Mutations</th>
<th>Exon 8</th>
<th>Exon 9</th>
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</table>
• What causes GIST?
• Where do GISTs come from (cell types)?
• What is “KIT”?

• How do “TKI” drugs (such as Gleevec) work?
• The “new generation” of GIST drugs
Farag et al., Revolutions in treatment options in GISTs: the latest updates, Curr. Treat. Options Oncol. 2020

The treatment of advanced GIST is rapidly evolving with the development of novel molecular compounds such as avapritinib and ripretinib ...

The availability of over five lines of treatment for patients with advanced GIST is likely to completely shift the current second-line and third-line treatment options ...

For GIST patients with tumours harbouring a D842V mutation in PDGFR exon 18, avapritinib ... will become first-line therapy for this molecular subgroup.

For second- and third-line treatment, results are awaited of a number of clinical trials. However, second-line and further treatment could potentially be tailored depending on secondary mutations found in imatinib-resistant GISTs. ...
“Bespoke” TKI drugs for GIST

Qinlock™ (ripretinib; DCC 2618)
Deciphera Pharmaceuticals

May 15, 2020: FDA approved ripretinib for adult patients with advanced GIST who have received prior treatment with three or more kinase inhibitors, including imatinib.
Ripretinib is a “switch-pocket” inhibitor; binds to KIT and prevents the protein from switching into its “active” conformation.

Imatinib cuts the fuel line to the engine; ripretinib jams the piston.
Qinlock™ (ripretinib) 50 mg tablets

Dhillon, S., Drugs (2020)
“Bespoke” TKI drugs for GIST

AYVAKIT™ (avapritinib; BLU-285)

Cambridge, Mass., June 29, 2020

Blueprint Medicines Corporation today announced that The Lancet Oncology published data from the NAVIGATOR clinical trial showing an unprecedented overall survival rate for AYVAKIT™ (avapritinib) in patients with advanced PDGFR D842V mutant GIST.

Michael Heinrich, M.D., Professor of Medicine at Oregon Health & Science University and primary author of the paper, said:

“It's tremendously rewarding to be able to offer - for the first time - a highly effective treatment option to my patients with PDGFR D842V mutant GIST.”

“AYVAKIT has become the new standard of care for patients with unresectable or metastatic GIST harboring a PDGFR exon 18 mutation,” said Andy Boral, M.D., Ph.D., Chief Medical Officer at Blueprint.
“The results of NAVIGATOR highlight the crucial role of gene testing in the diagnosis and treatment of GIST. Despite decreasing costs of single gene and next-generation sequencing panels, frequencies of genetic testing remain low among newly diagnosed patients with GIST. ... Avapritinib could potentially improve the disease course of metastatic PDGFR D842V-mutant GIST, which previously had a dire prognosis. Failure to treat this subgroup as a result of inadequate gene profiling represents a truly missed opportunity.

These findings provide additional evidence supporting the paradigm shift towards precision oncology and emphasise the usefulness of genomic sequencing in the personalisation of therapy for improving outcomes for patients with GIST.”

Nguyen, Banerjee, and Sicklick, Moving gastrointestinal stromal tumours towards truly personalised precision therapy, *Lancet Oncology*, July 1, 2020

TRK fusions are oncogenic drivers of various adult and paediatric cancers. The first-generation TRK inhibitors, larotrectinib and entrectinib, were granted landmark, tumour-agnostic regulatory approvals ... in 2018 and 2019, respectively. Brisk and durable responses are achieved. ...

These next-generation drugs are currently available in the clinic and proof-of-concept responses have been reported.

(ETV6-NTRK mutations are known - but very rare - driver mutations in GIST.)
On the horizon .... AZD3229


We report the discovery and pharmacological characterization of AZD3229, a potent and selective small-molecule inhibitor of KIT and PDGFRα designed to inhibit a broad range of primary and imatinib-resistant secondary mutations seen in GIST. In engineered and GIST-derived cell lines, AZD3229 is 15 to 60 times more potent than imatinib in inhibiting KIT primary mutations ... AZD3229 has a superior potency and selectivity profile [and] has the potential to be a best-in-class inhibitor for clinically relevant KIT/PDGFRα mutations in GIST.
David Josephy
Life Raft Group Canada

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