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.

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

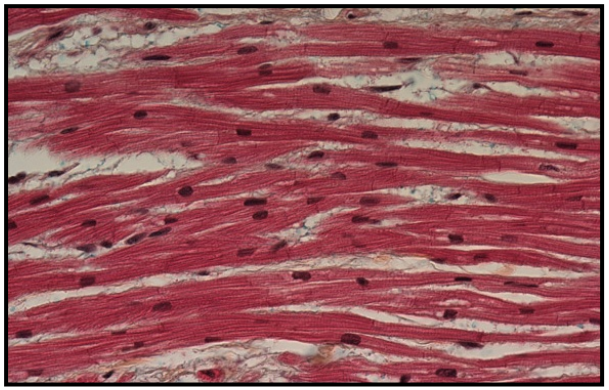
Ricci, R., Syndromic gastrointestinal stromal tumors, *Hered. Cancer Clin. Pract.*, 2016

This presentation is limited to sporadic GIST.

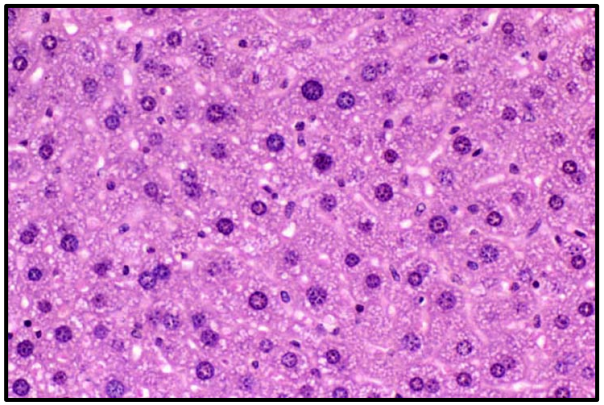
- What causes GIST?
- **Where do GISTs come from (cell types)?**
- What is “KIT”?

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

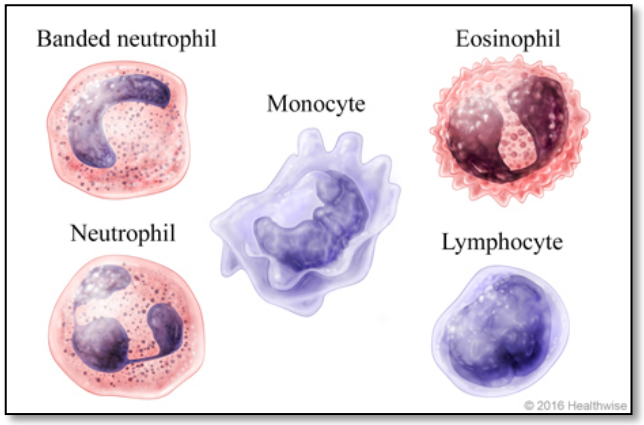
There are hundreds of different types of cells in the body.



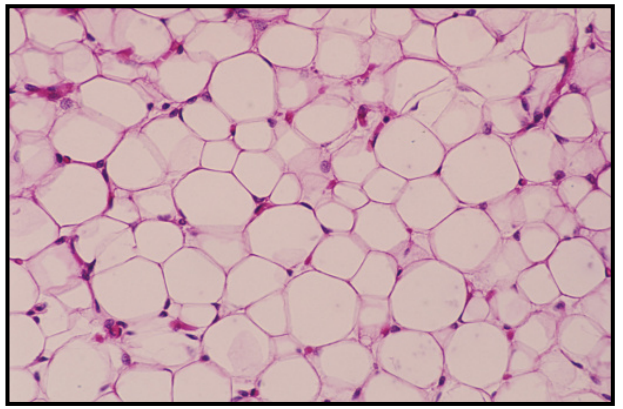
cardiomyocytes (heart)



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

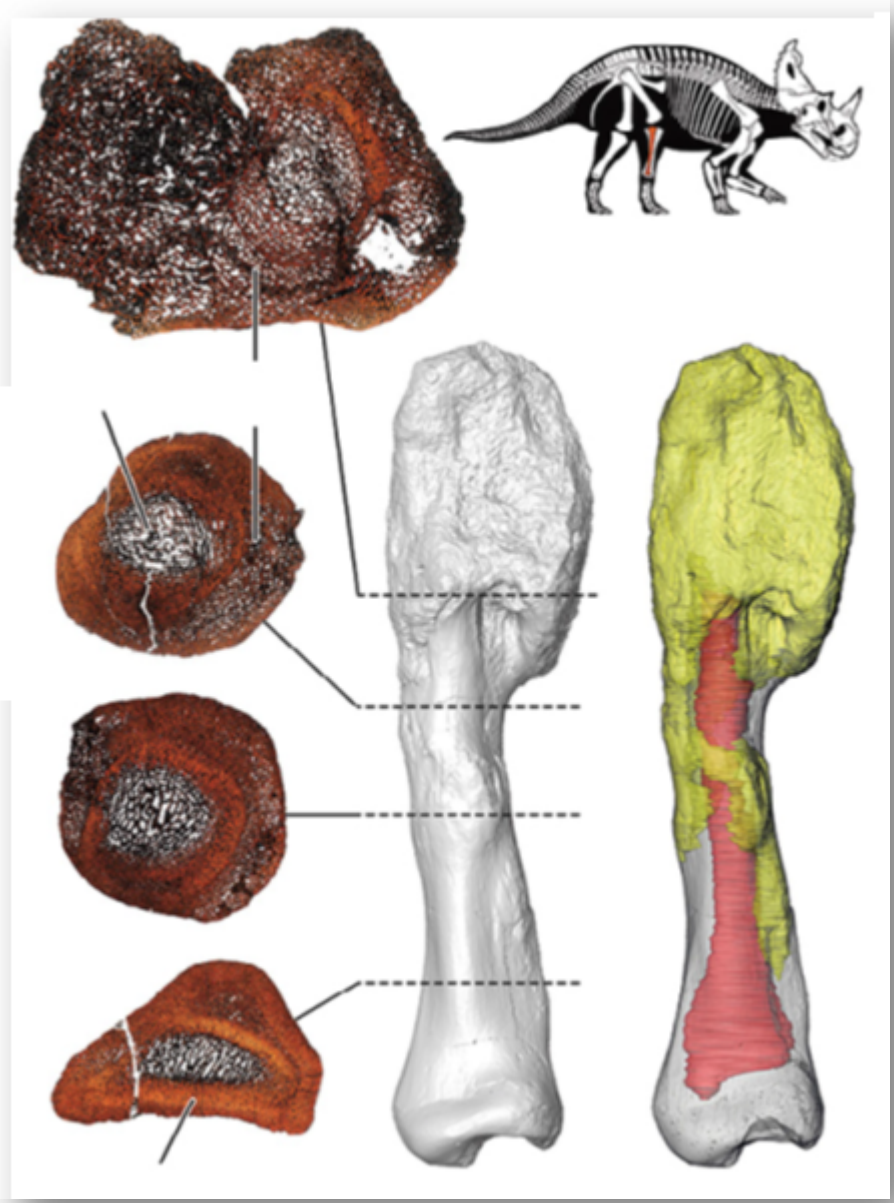
Carcinomas – the most common cancers – skin, colon, lung, prostate, breast, etc. - arise in epithelial ("lining") cells.

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

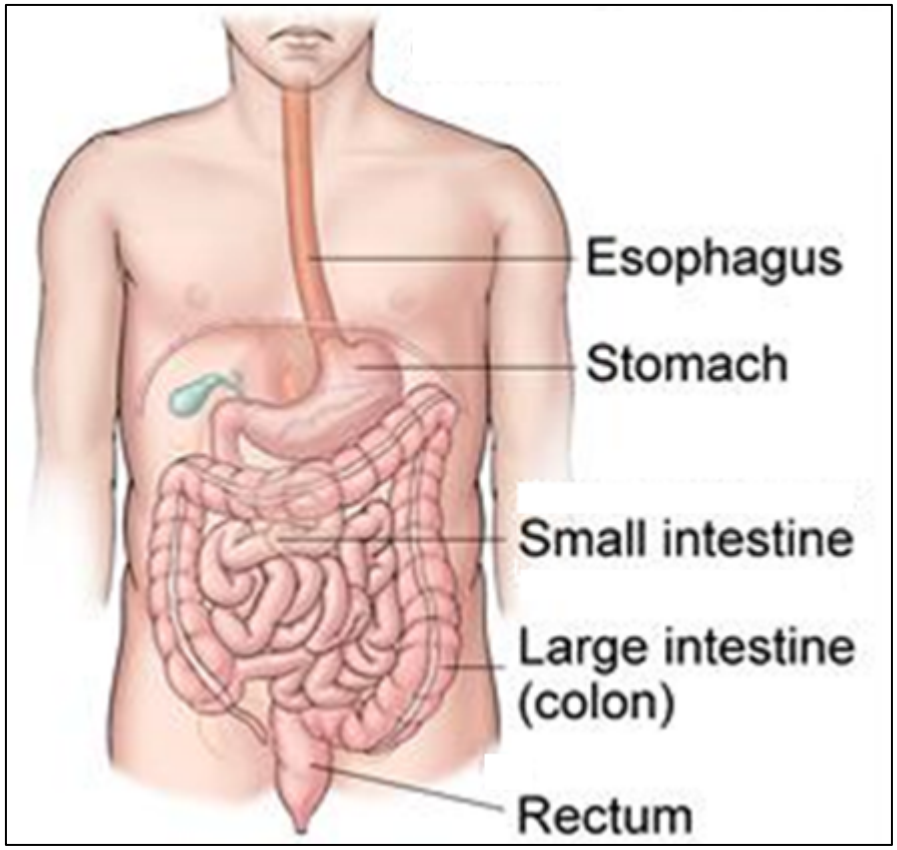
Sarcomas are rare (about 1% of human cancers).

Treating sarcomas is a sub-specialty among oncologists; treating GISTs is a sub-sub-specialty!

An osteosarcoma in a horned dinosaur, 77 million years ago; Ekhtiari *et al.*, *Lancet Oncology,* Aug. 2020.



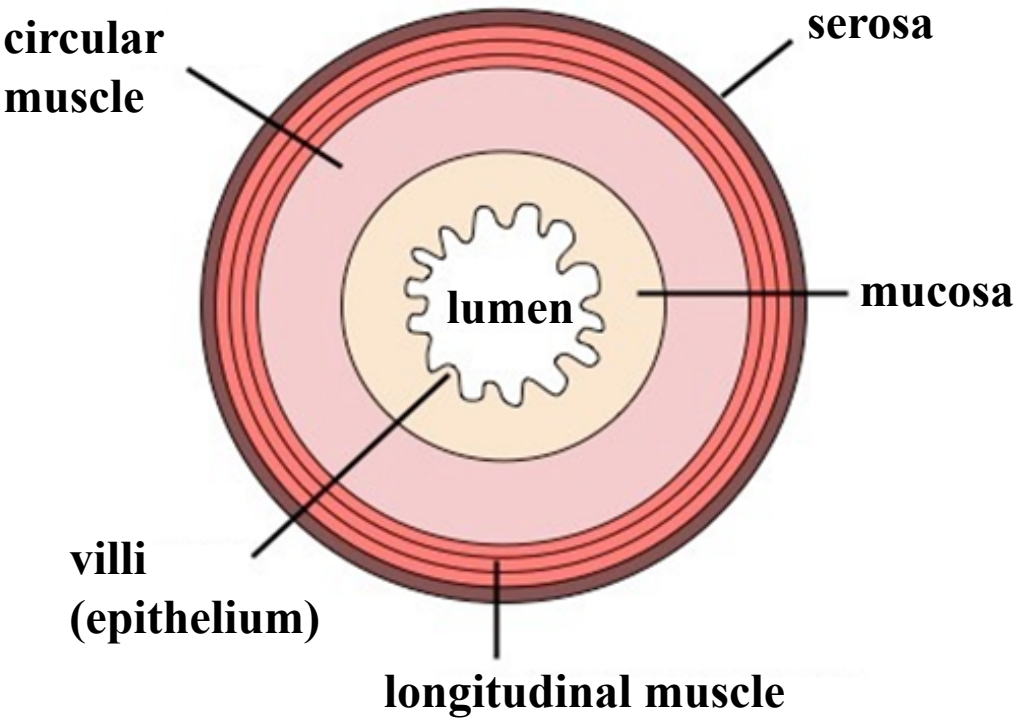
The Gastrointestinal Tract: a 5-metre-long tube.

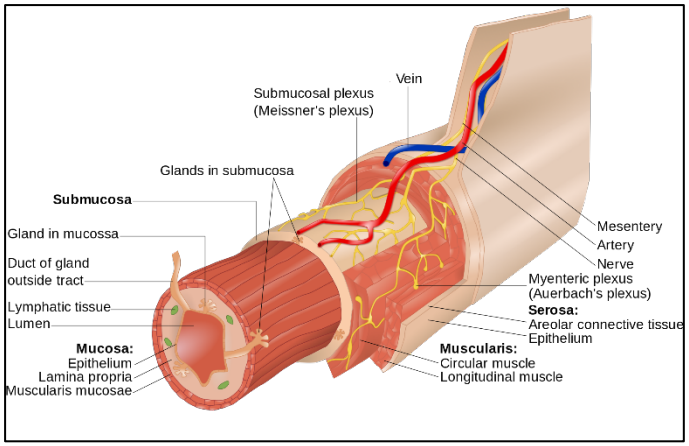


GI carcinomas are common
(stomach cancer, colon cancer, etc.)

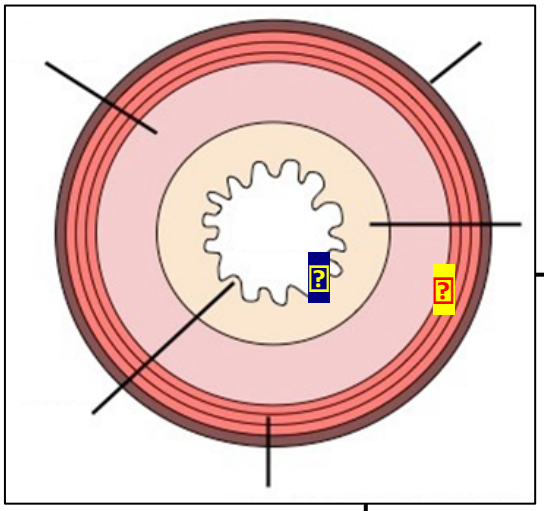
GIST (sarcoma) is rare.

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

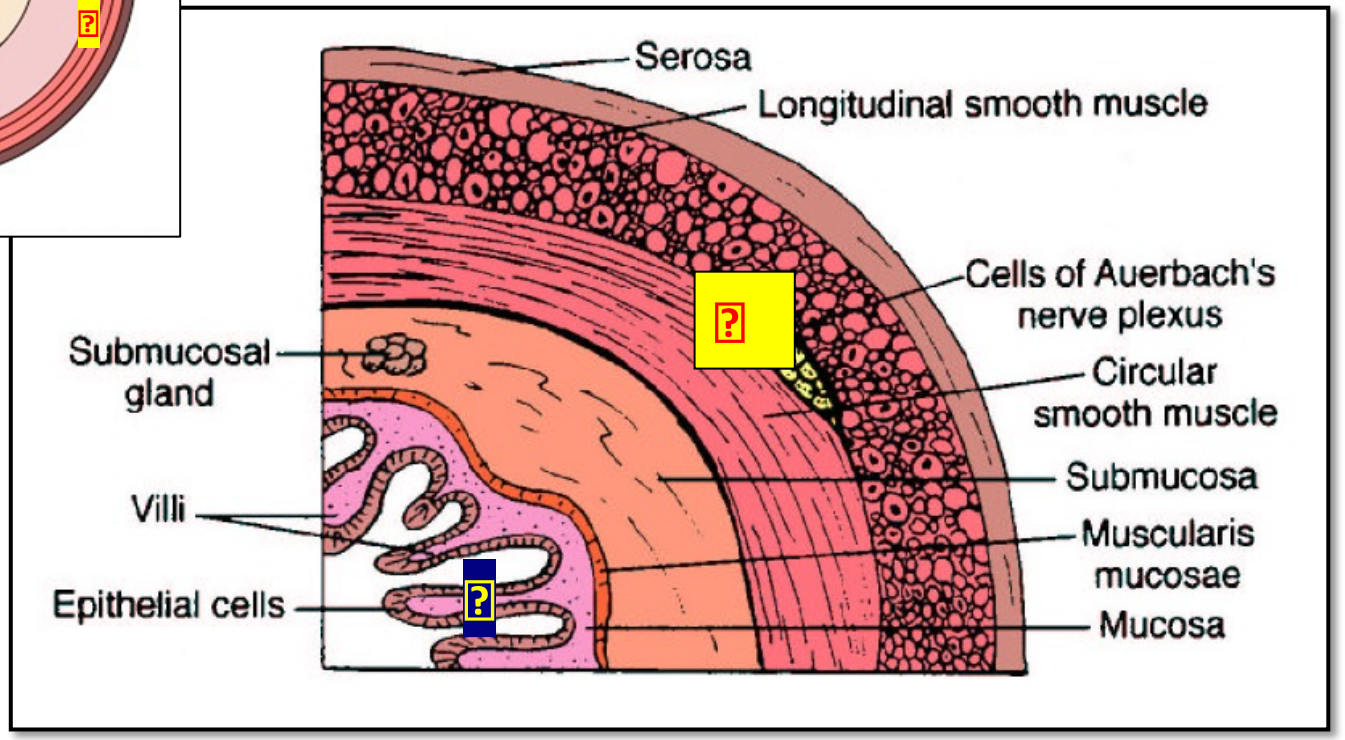




Cross-section of the GI tract

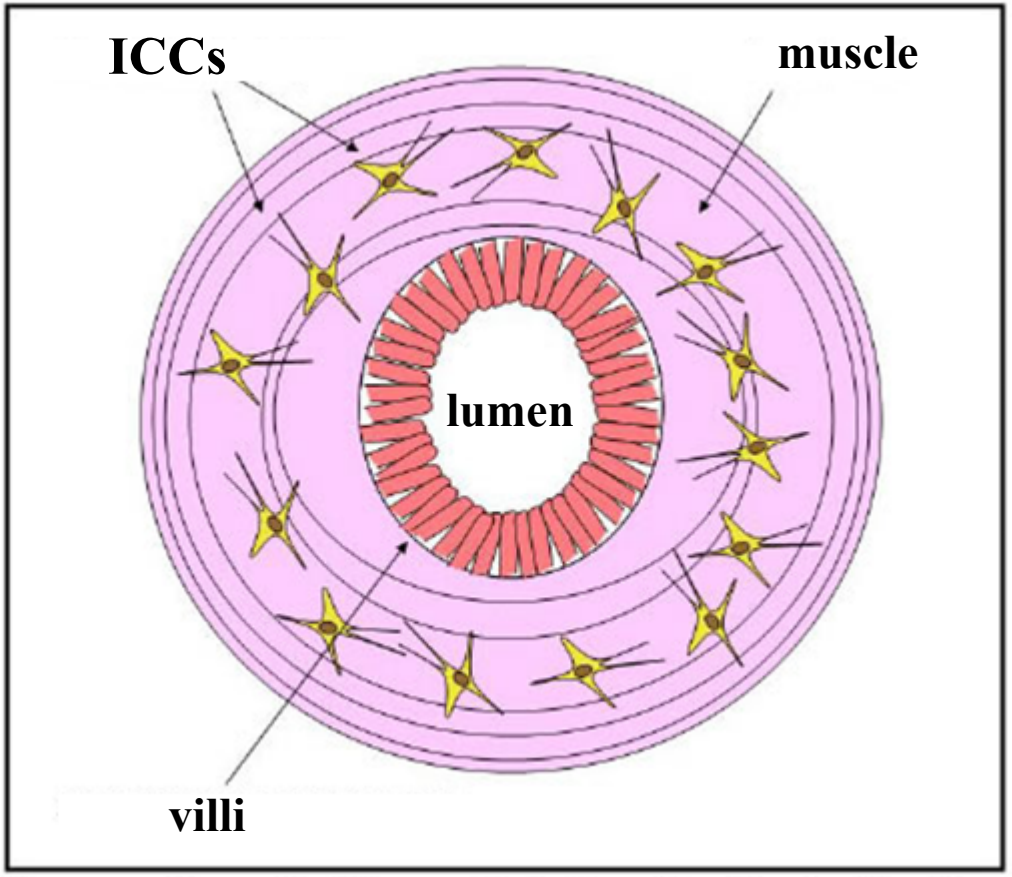


 carcinomas start in the epithelial lining (the body's "outside" surface)



 GISTs (sarcomas) start in the muscular wall

Interstitial Cells of Cajal: the cells where GISTs start; the "pacemaker" cells that coordinate GI peristalsis.

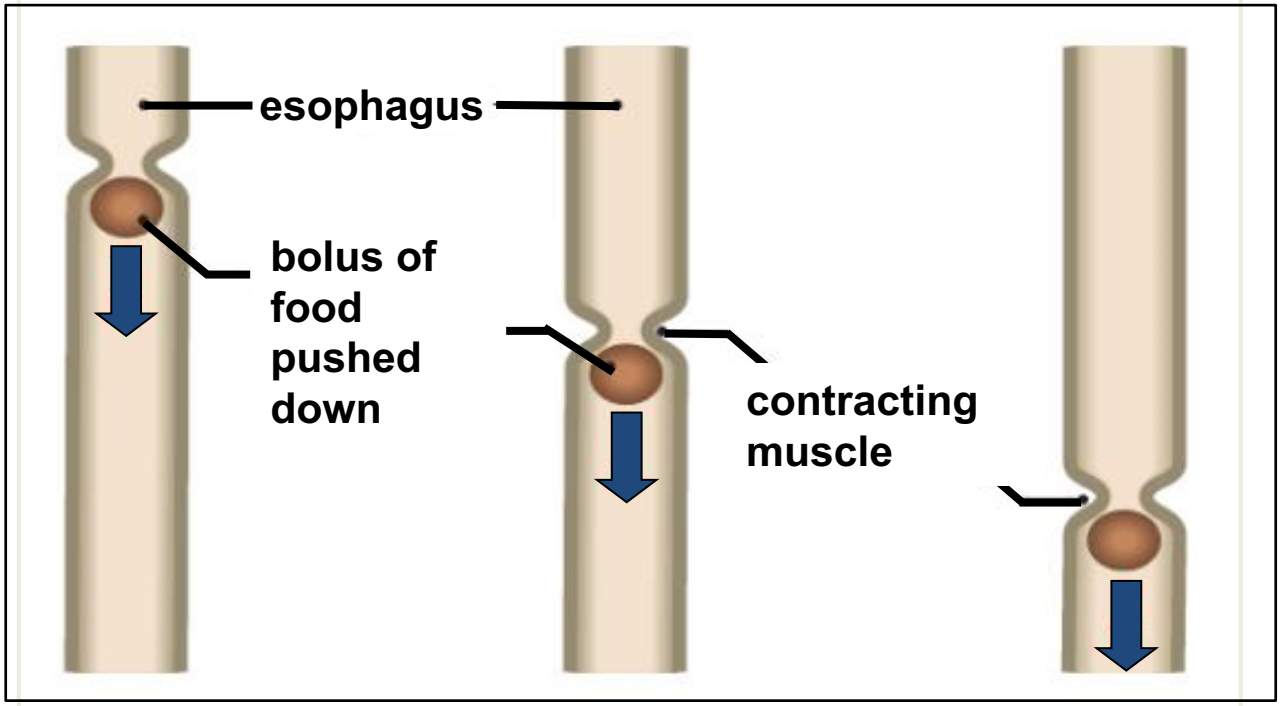


(1852-1934)

liferaftgroup.org/2009/06/interstitial-cells-of-cajal-what-are-they-and-why-should-you-care/

Huizinga and Chen, Interstitial cells of Cajal: update on basic and clinical science, *Curr. Gastroenterol. Rep.* (2014)

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

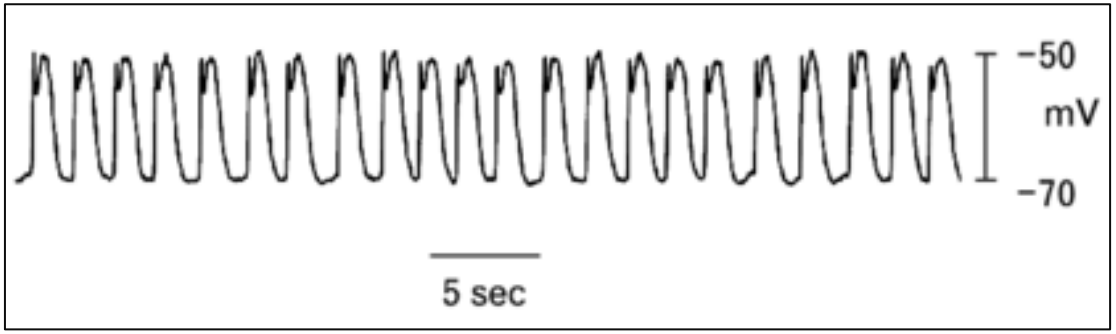


The coxswain keeps the crew's muscles synchronized.

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.

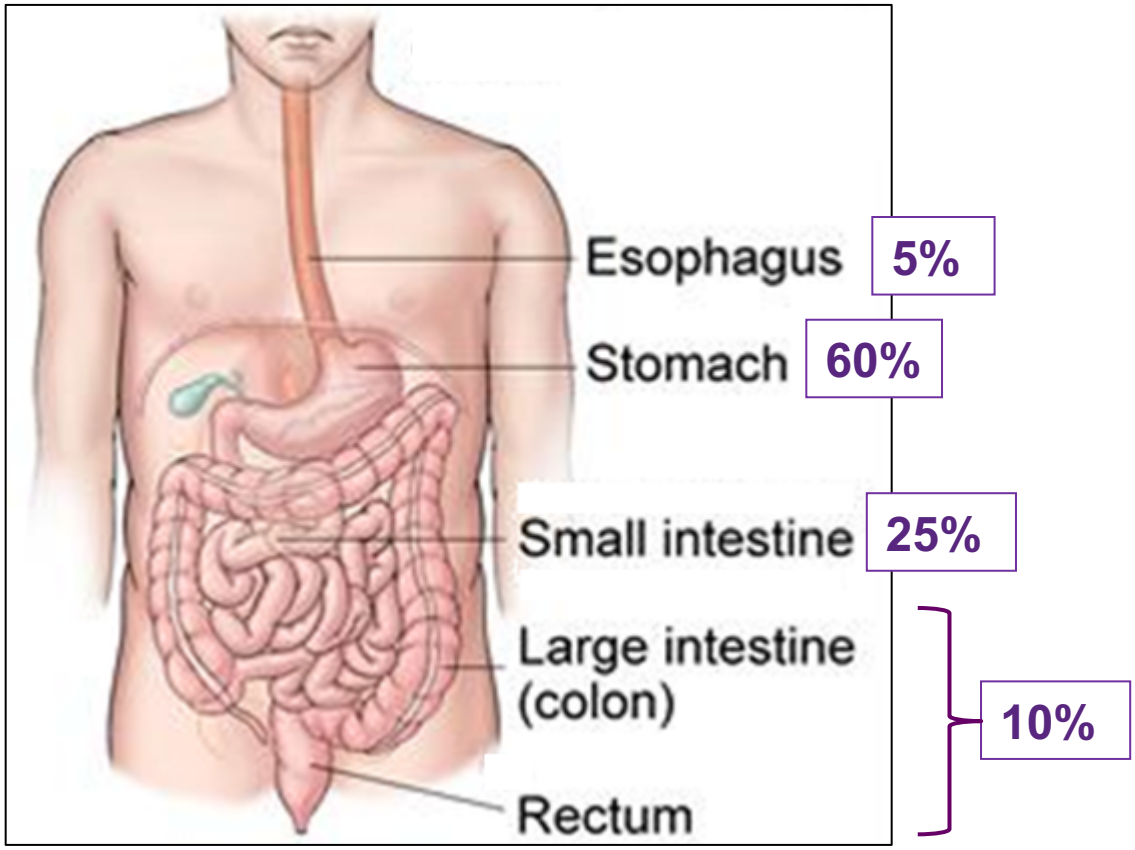
ICCs are the cells where GISTs start.

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".

An Englishman in New York is still an Englishman.

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- How do “TKI” drugs (such as Gleevec) work?
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The Molecular Biology of GIST …

and a crash course in Molecular Biology!

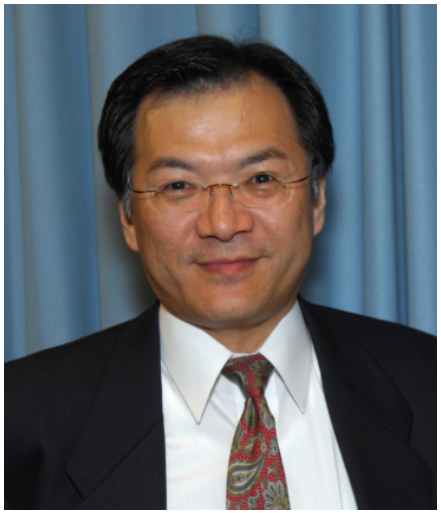
The GIST-KIT connection: the 1998 breakthrough

that revolutionized GIST diagnosis and treatment.

- GIST cells almost always express a protein called "KIT"

  (very few other cells in the body do so)

- In most cases of GIST, the *KIT* gene is mutated, producing an aberrant form of KIT protein that "drives" cell division and therefore drives the cancer.



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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.

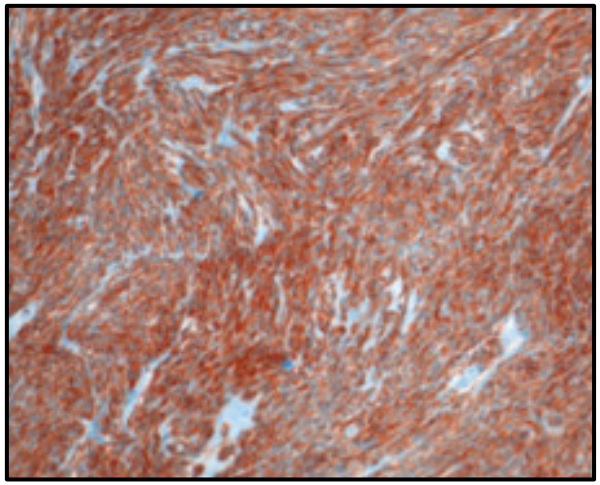
KIT ("c-Kit" or "CD117")

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.



Di Vizio *et al.*, 2008

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

SURGICAL PATHOLOGY REPORT

Immunohistochemical studies

KIT (CD117): positive

DOG1 (ANO1): positive

Other(specify): CD34: positive; Smooth muscle actin: negative; desmin: negative; S100: negative.

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

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

*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 *PDGFR*? mutant GIST.

RESULTS
NGS Panel: Actionable variant(s) detected
--Variant 1
Gene: PDGFRA
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.

CLASS 1 Variants: *Variants actionable in the disease site in which they have been identified.*

PDGFRA (NM_006206.4) c.2525A>T (p.Asp842Val)

The PDGFRA gene is recurrently mutated in GIST (mycancergenome.org). The p.Asp842Val variant in PDGFRA is associated with resistance to treatment with tyrosine kinase inhibitors imatinib and sunitinib (mycancergenome.org; PMID: 15928335). Preclinical studies suggest that this variant is responsive to dasatinib, but not sorafenib or nilotinib (PMID: 18794084).

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_033360.3], NRAS [NM_002524.3], PIK3CA [NM_006218.2]

Lung cancer: AKT1 [NM_001014432.1], BRAF [NM_004333.4], EGFR [NM_005228.3], ERBB2 [NM_ 004448.3], KRAS [NM_033360.3], PIK3CA [NM_006218.2], RET [NM_020975.4], TP53 [NM_000546.5]

GIST: KIT [NM_000222.2], PDGFRA [NM_006206.4]

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.

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.

Genes and Proteins

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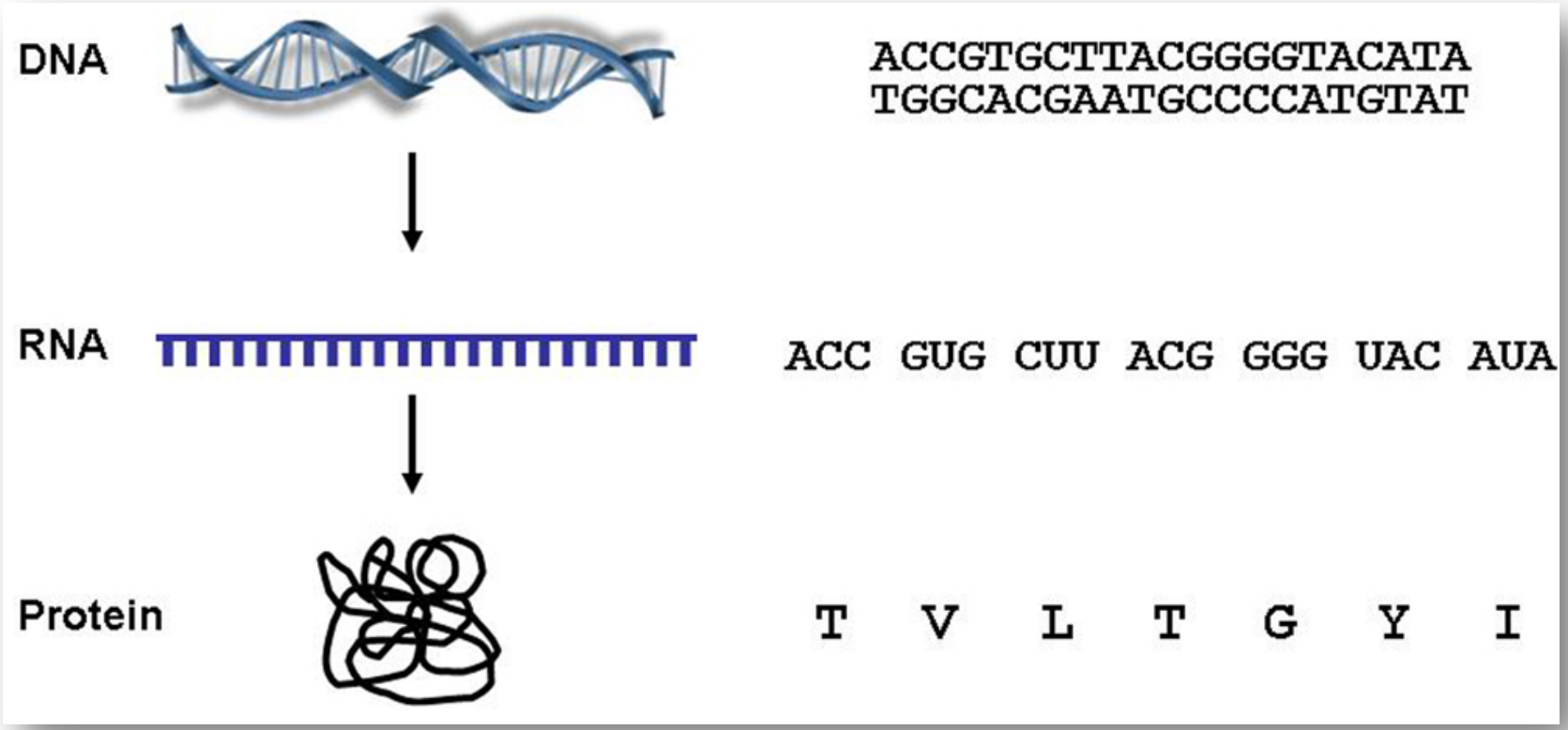

Proteins

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
etc.

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 LCVLLLLLRV QTGSSQPSVS PGEPSPPSIH PGKSDLIVRV GDEIRLLCTD
61  PGFVKWTFEI LDETNENKQN EWITEKAEAT NTGKYTCTNK HGLSNSIYVF VRDPAKLFLV
121 DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH
181 RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS
241 SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN
301 VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE
361 DYPKSENESN IRYVSELHLT RLKGTEGGTY TFLVSNSDVN AAIAFNVYVN TKPEILTYDR
421 LVNGMLQCVA AGFPEPTIDW YFCPGTEQRC SASVLPVDVQ TLNSSGPPFG KLVVQSSIDS
481 SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMMCIIV
541 MILTYKYLQK PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS FGKTLGAGAF
601 GKVVEATAYG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC
661 TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE
721 YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM
781 AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGNAR LPVKWMAPES
841 IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAEMY
901 DIMKTCWDAD PLKRPTFKQI VQLIEKQISE STNHIYSNLA NCSPNRQKPV VDHSVRINSV
961 GSTASSSQPL LVHDDV
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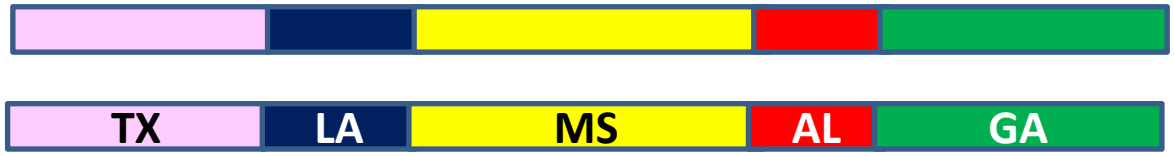
Protein structure: Exons*

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*

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121 DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH
181 RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS
241 SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN
301 VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE
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601 GKVVEATAYG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC
661 TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE
721 YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM
781 AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGNAR LPVKWMAPES
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      1                                                2
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                                                       3
61    PGFVKWTFEI LDETNENKQN EWITEKAEAT NTGKYTCTNK HGLSNSIYVF VRDPAKLFLV
                                          4
121   DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH
                                    5
181   RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS
                                6                                   7
241   SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN
                                                                    8
301   VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE
                                                9
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                                                10
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                                                10
481   SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMMCIIV
                                  11                                12
541   MILTYKYLQK PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS FGKTLGAGAF
                                          13
601   GKVVEATAYG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC
          14                                                        15
661   TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE
                                        16
721   YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM
                17                                      18
781   AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGNAR LPVKWMAPES
                                    19                              20
841   IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAEMY
                                              21
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                                          4
181 RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS
                    5
241 SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN
                6                                                  8
301 VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE
                  7
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421 LVNGMLQCVA AGFPEPTIDW YFCPGTEQRC SASVLPVDVQ TLNSSGPPFG KLVVQSSIDS
                                                10
481 SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMMCIIV
                11                                                 12
541 MILTYKYLQK PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS FGKTLGAGAF
                                          13
601 GKVVEATAYG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC
          14                                                      15
661 TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE
                                      16
721 YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM
            17                                          18
781 AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGNAR LPVKWMAPES
                                    19                            20
841 IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAEMY
                                          21
901 DIMKTCWDAD PLKRPTFKQI VQLIEKQISE STNHIYSNLA NCSPNRQKPV VDHSVRINSV
961 GSTASSSQPL LVHDDV
```
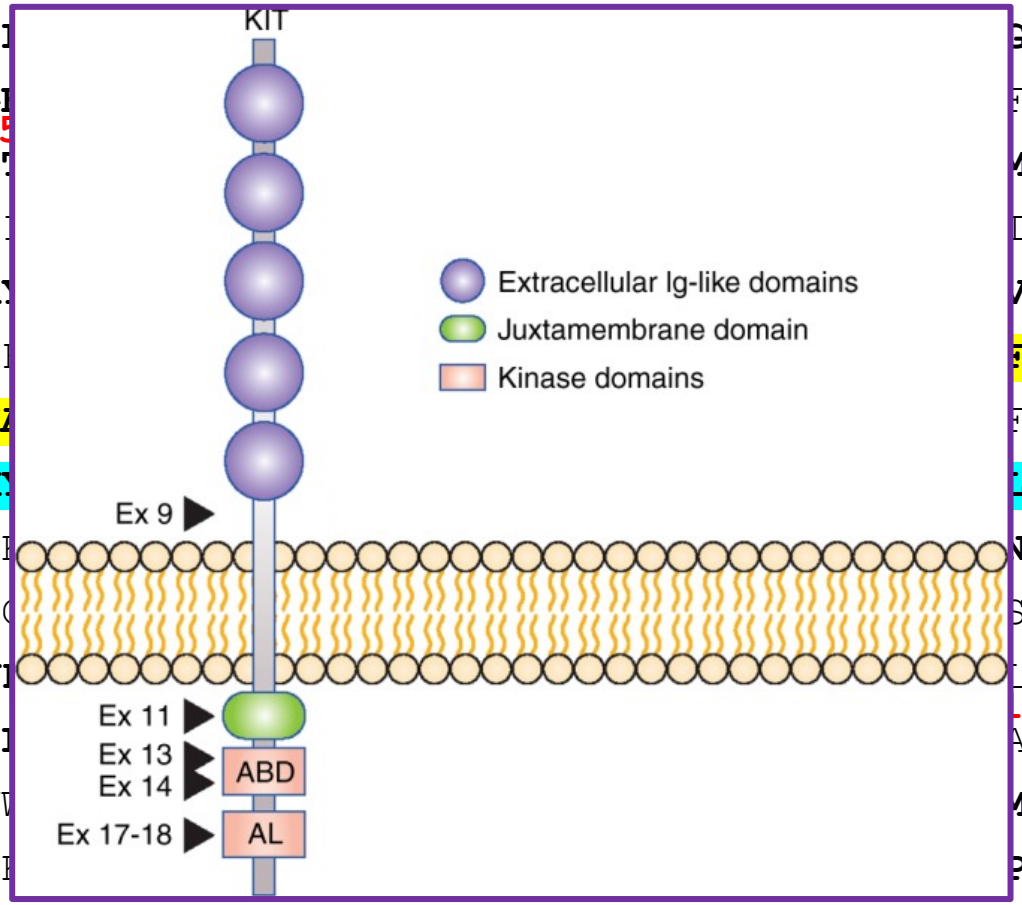
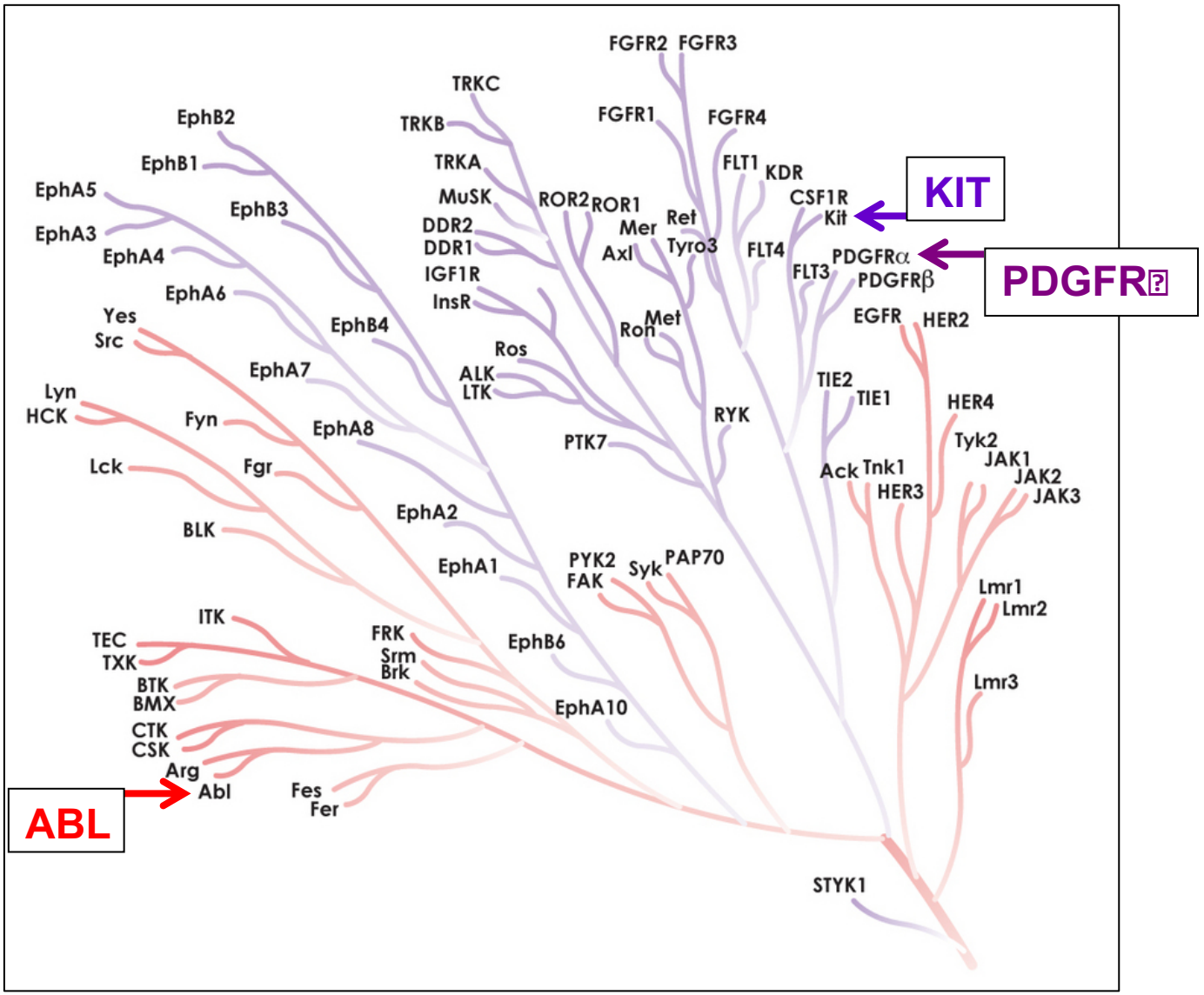

The KIT protein: 21 exons



KIT is one member of a large family of related proteins. PDGFR□ is a "sister"; ABL is a "distant cousin".

human tyrosine kinases



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.

KIT mutations

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).

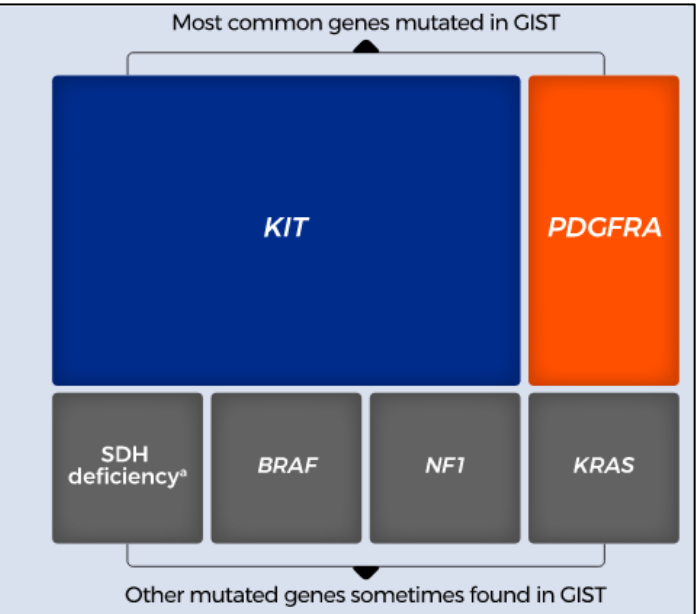
www.mutationmatters.com (Blueprint Medicines)

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*, KRAS proto-oncogene, GTPase; *NF1*, neurofibromin 1; *PDGFRA*, platelet-derived growth factor receptor alpha; SDH, succinate dehydrogenase.

[a] SDH deficiency refers to a decrease in succinate dehydrogenase (SUX-sin-ate dee-high-DRAW-jen-ase), a protein. The decrease can develop from mutations in specific genes.



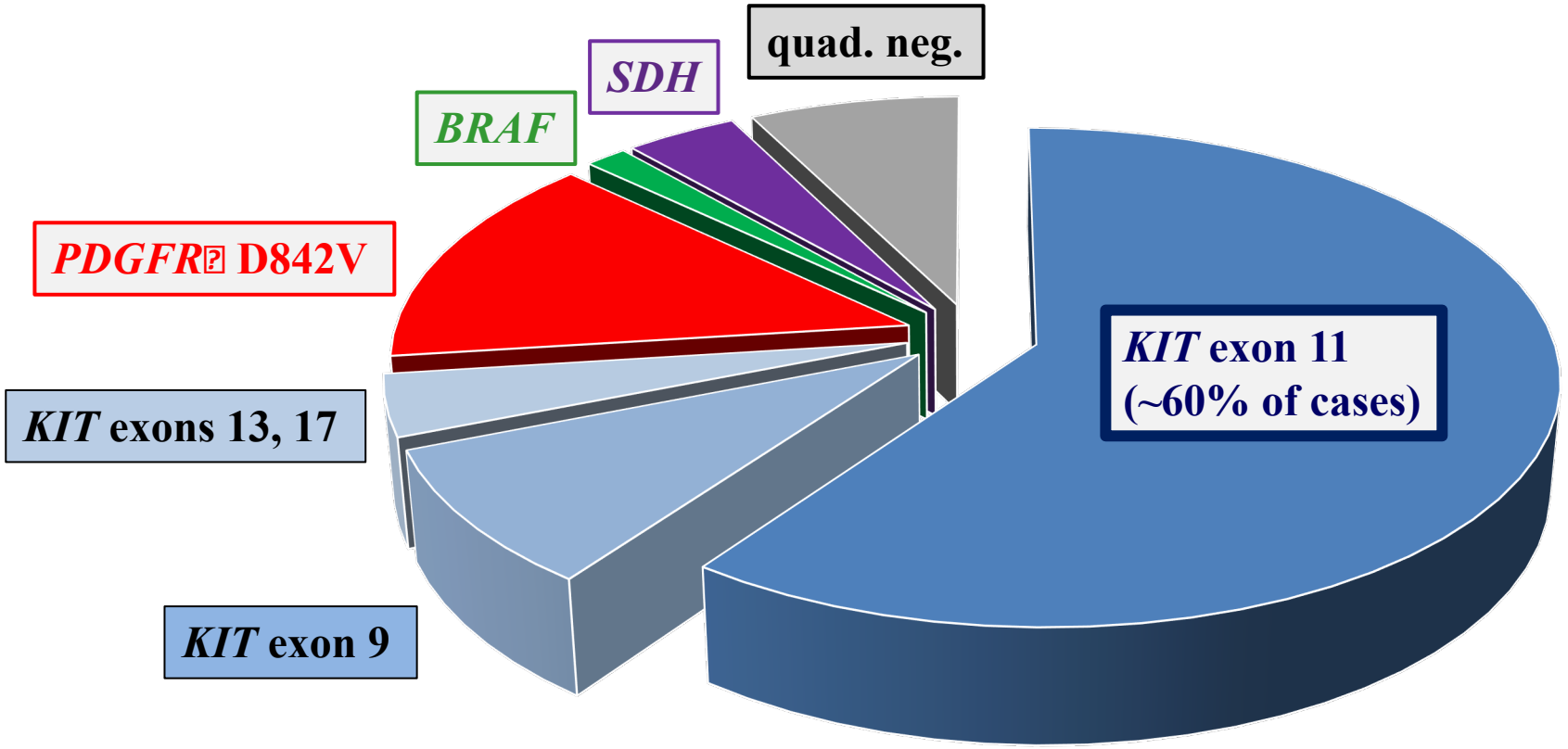
In the United States, 3 of 4 people with GIST may not be tested for mutations.

Reference: Florindez J, Trent J. Low frequency of mutation testing in the United States: an analysis of 3866 GIST patients. *Am J Clin Oncol*. Published online Jan 3, 2020. doi:10.1097/COC.0000000000000659

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

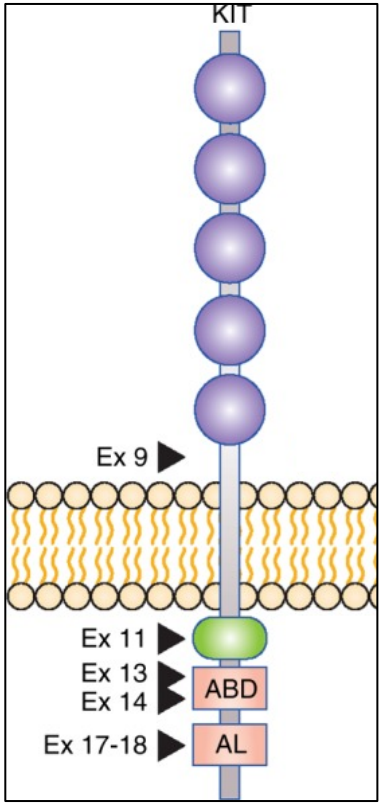
The KIT protein: 21 exons

Exons 9 and 11 are the regions of the KIT gene/ protein where most of the primary mutations in GISTs are found.

```
1   MRGARGAWDF LCVLLLLLRV QTGSSQPSVS PGEPSPPSIH PGKSDLIVRV GDEIRLLCTD
61  PGFVKWTFEI LDETNENKQN EWITEKAEAT NTGKYTCTNK HGLSNSIYVF VRDPAKLFLV
121 DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH
181 RLCLHCSVDQ EGKSVLSEKF ILKVRPAFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS
241 SVYSTWKREN SQTKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN
301 VTTTLEVVDK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE
361 DYPKSENESN IRYVSELHL[Exon 9]GTY TFLVSNSDVN AAIAFNVYVN TKPEILTYDR
421 LVNGMLQCVA AGFPEPTID[Exon 9]QRC SASVLPVDVQ TLNSSGPPFG KLVVQSSIDS
481 SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMMCIIV
541 [Exon 11]QK PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS FGKTLGAGAF
601 [Exon 11]YG LIKSDAAMTV AVKMLKPSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC
661 TIGGPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESSCSDSTNE
721 YMDMKPGVSY VVPTKADKRR SVRIGSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM
781 AFLASKNCIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGNAR LPVKWMAPES
841 IFNCVYTFES DVWSYGIFLW ELFSLGSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAEMY
901 DIMKTCWDAD PLKRPTFKQI VQLIEKQISE STNHIYSNLA NCSPNRQKPV VDHSVRINSV
961 GSTASSSQPL LVHDDV
```

Exon 11 encodes the “juxtamembrane” domain of the KIT protein.

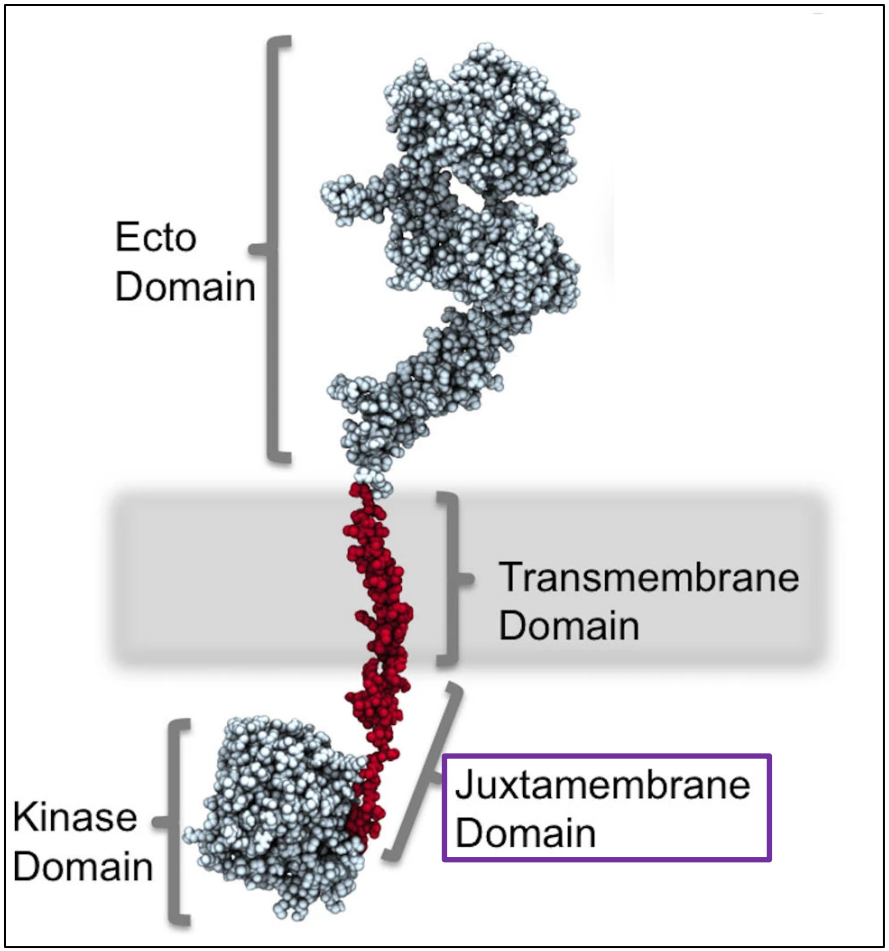
Exon 11 mutations cause a conformational change (“switch”) of KIT protein from its “inactive” to its “active” form, signaling the GIST cell to grow and divide.



Extracellular Ig-like domains

Juxtamembrane domain

Kinase domains



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 560th 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 557th and 558th amino acids in the KIT protein are absent.

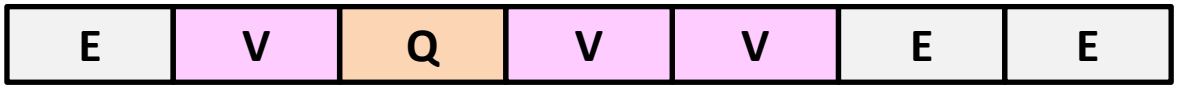
KIT-mutant GIST: examples

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

554

after mis-sense mutation V560D …

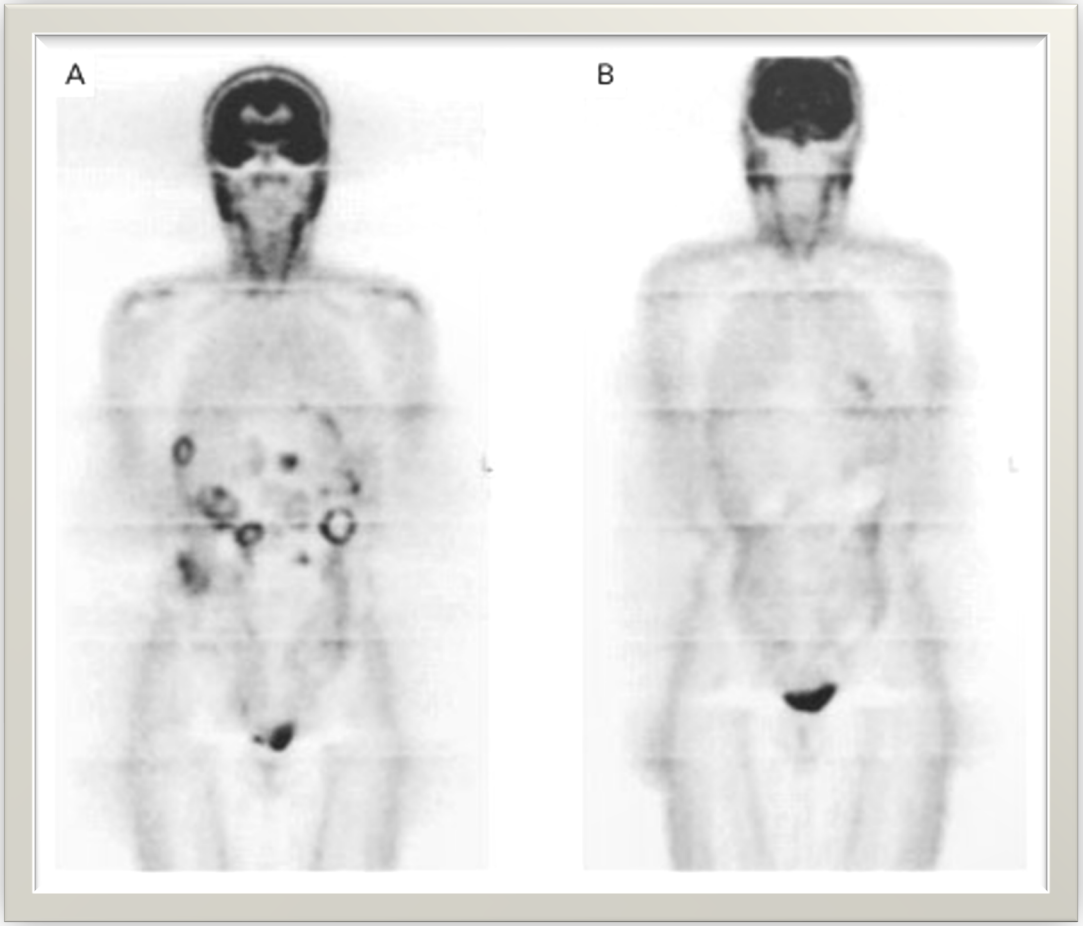
after deletion mutation W557_K558 …



- 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



Joensuu *et al.*, *N. Engl. J. Med.* 344: 1052-1056, 2001.

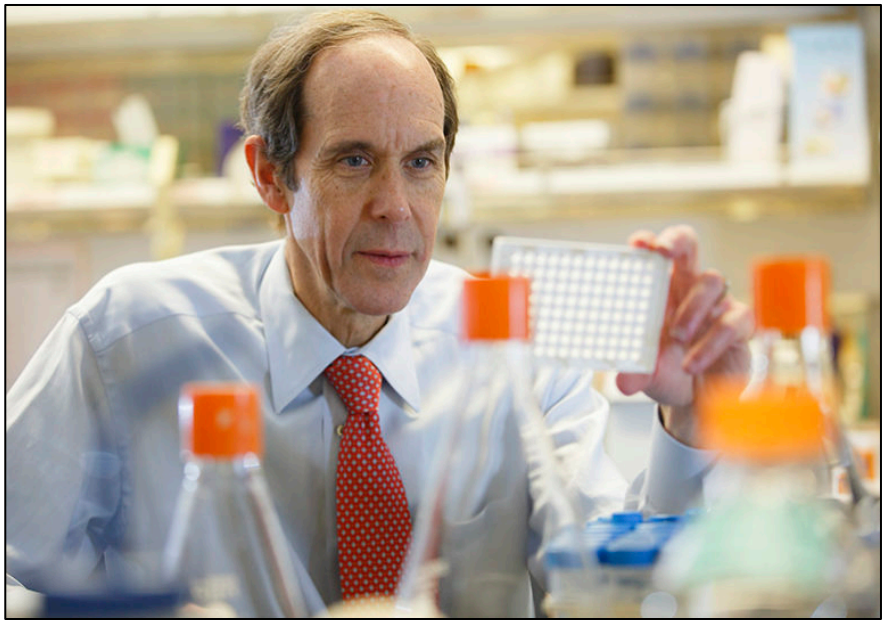
"Targeted" drugs for treating GIST

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*.

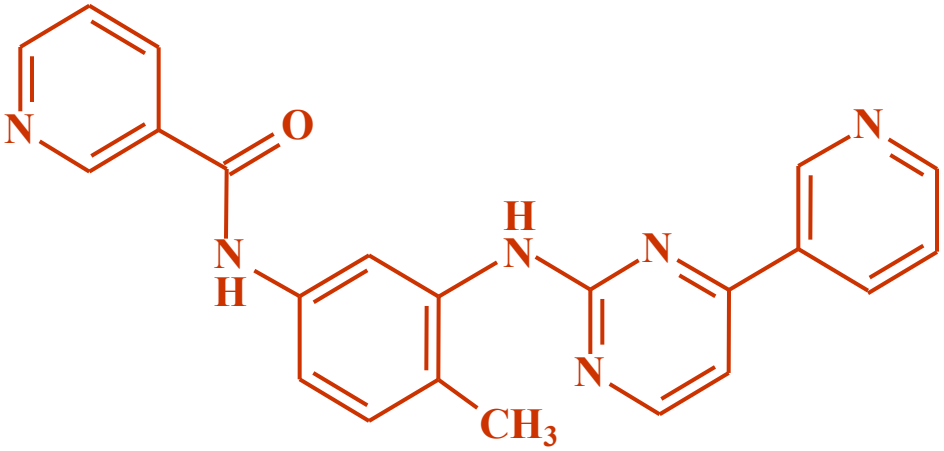
ABL is a "tyrosine kinase" enzyme. Drugs that inhibit (shut down) those enzymes are "tyrosine kinase inhibitors" (TKIs).

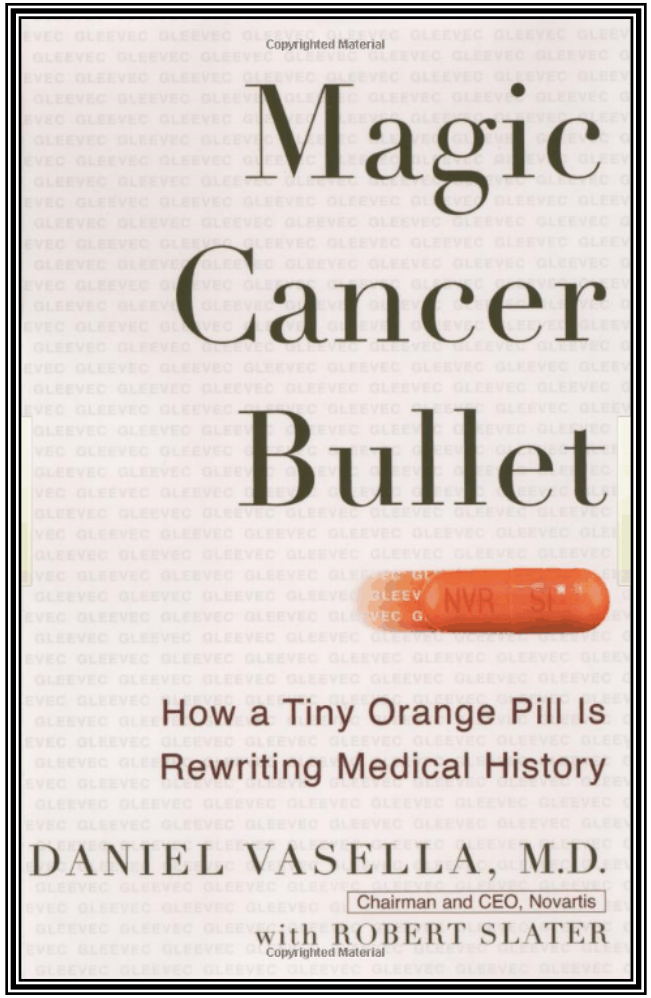


Brian J. Druker,
Oregon Health & Science University,
Portland, USA

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)

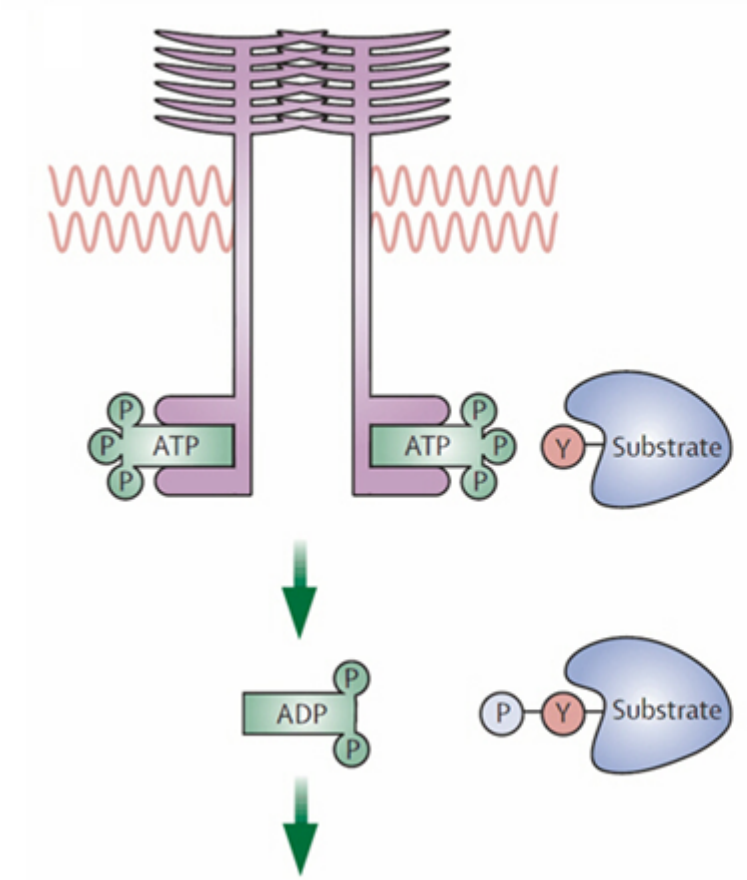
Third-line: Regorafenib (Stivarga - Bayer; 2013)

(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.

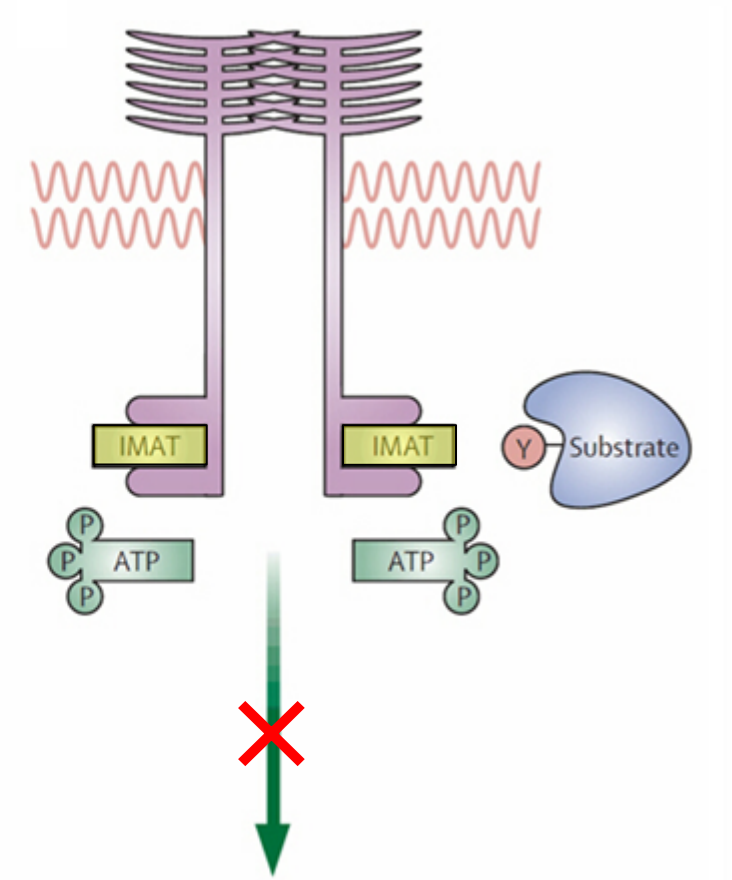
"Second generation" GIST drugs, such as ripretinib (to be discussed later), use different mechanisms of KIT inhibition.

untreated



KIT-activated signal transduction;
GIST proliferation and survival

imatinib



inhibition of KIT;
reduced GIST proliferation;
apoptosis (cell death)

Rubin *et al.*, *Lancet* 2007

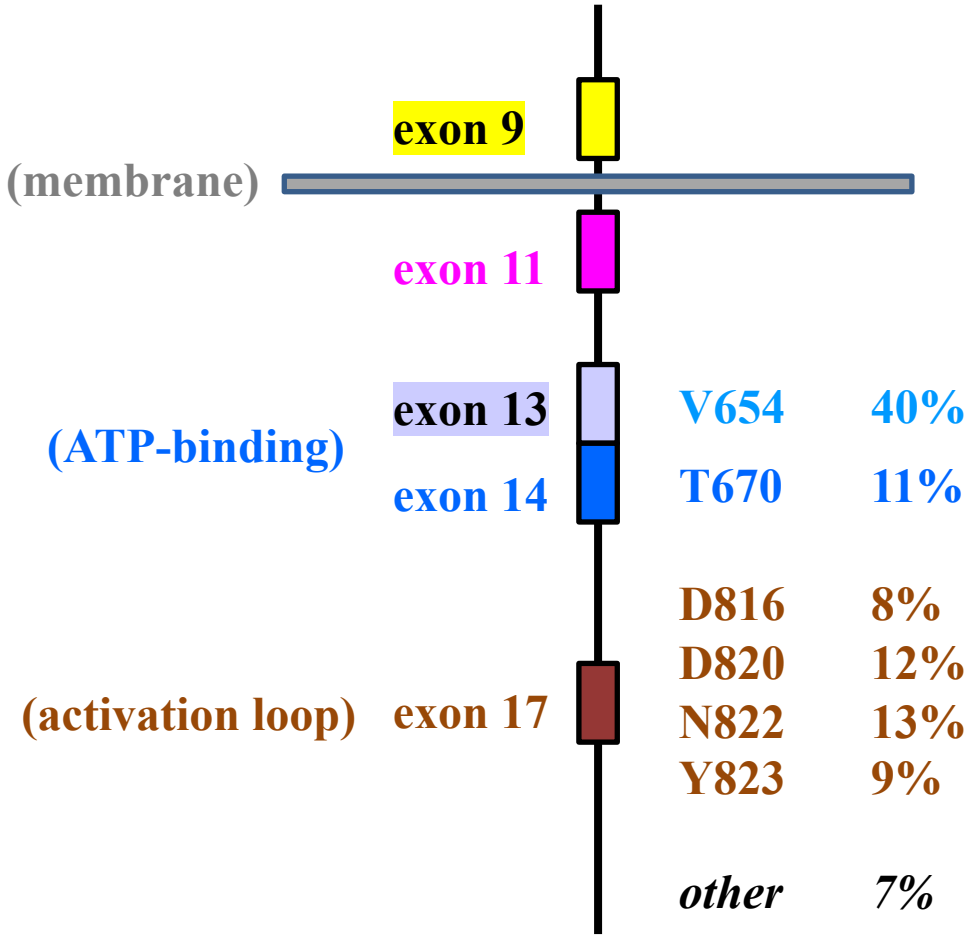
Despite the success of these drugs, more are needed:

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

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

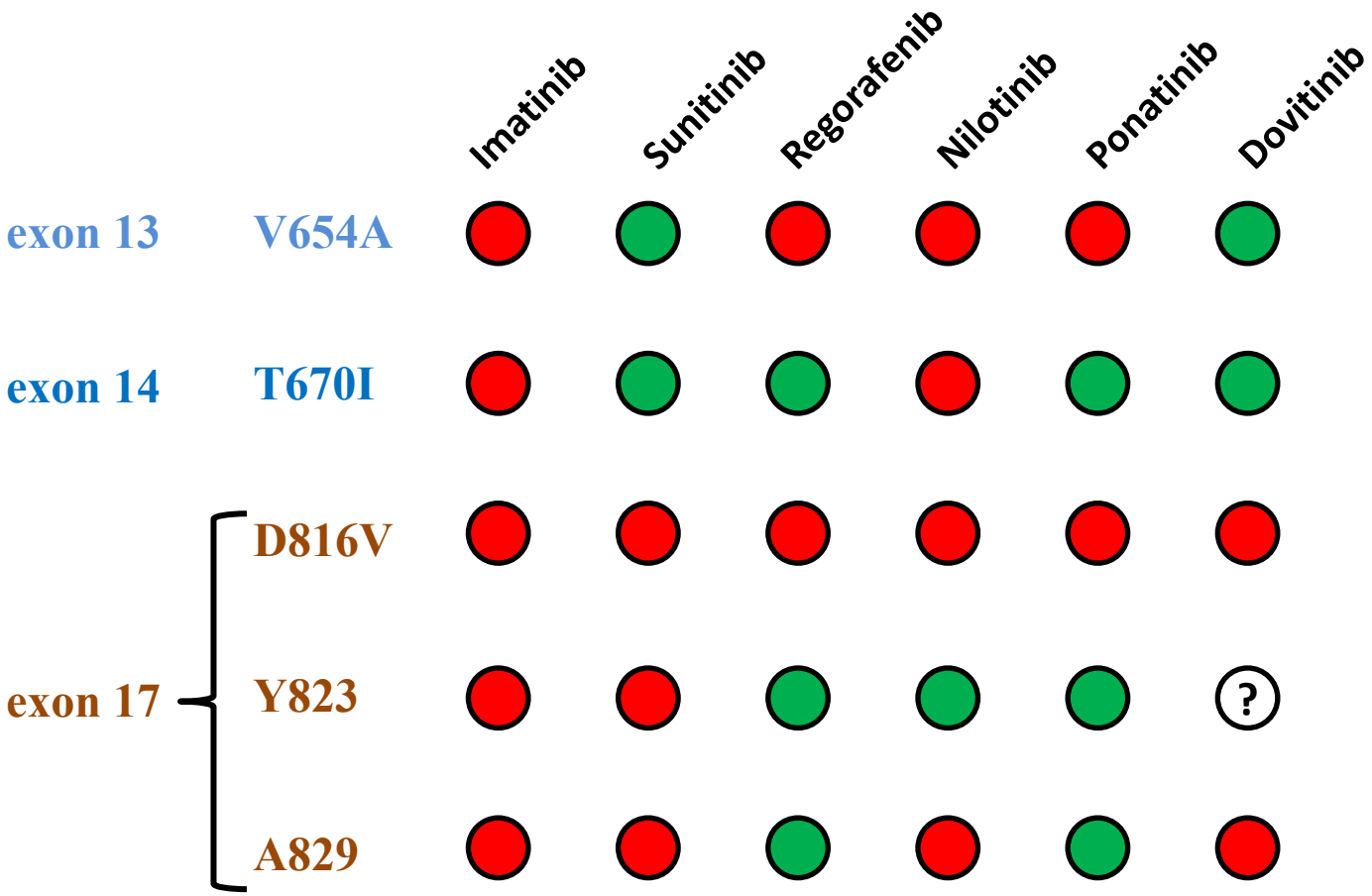
- **Imatinib halts the growth of most GISTs, but does not eliminate them; over time, GIST tumours tend to become imatinib-resistant, mainly due to additional mutations arising in the metastases.**

imatinib resistance: secondary mutations in *KIT*



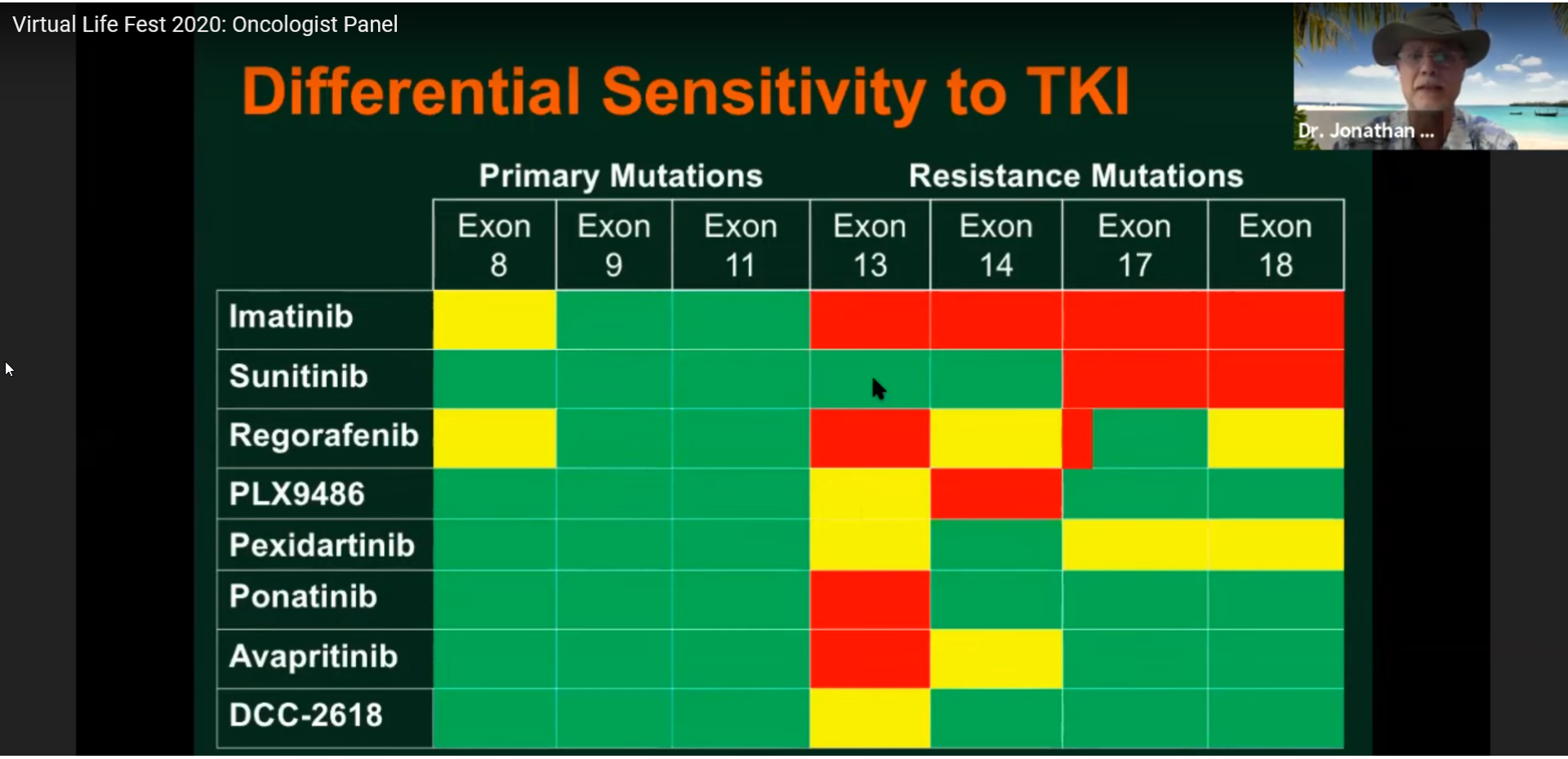
adapted from: Serrano, C. ... Heinrich, M.C. … and Fletcher, J.A., *Br. J. Cancer* 120: 612-620, 2019.

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



adapted from Serrano and Fletcher, *Oncotarget* 2019

July 2020 update by Dr. Jon Trent



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- **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. …

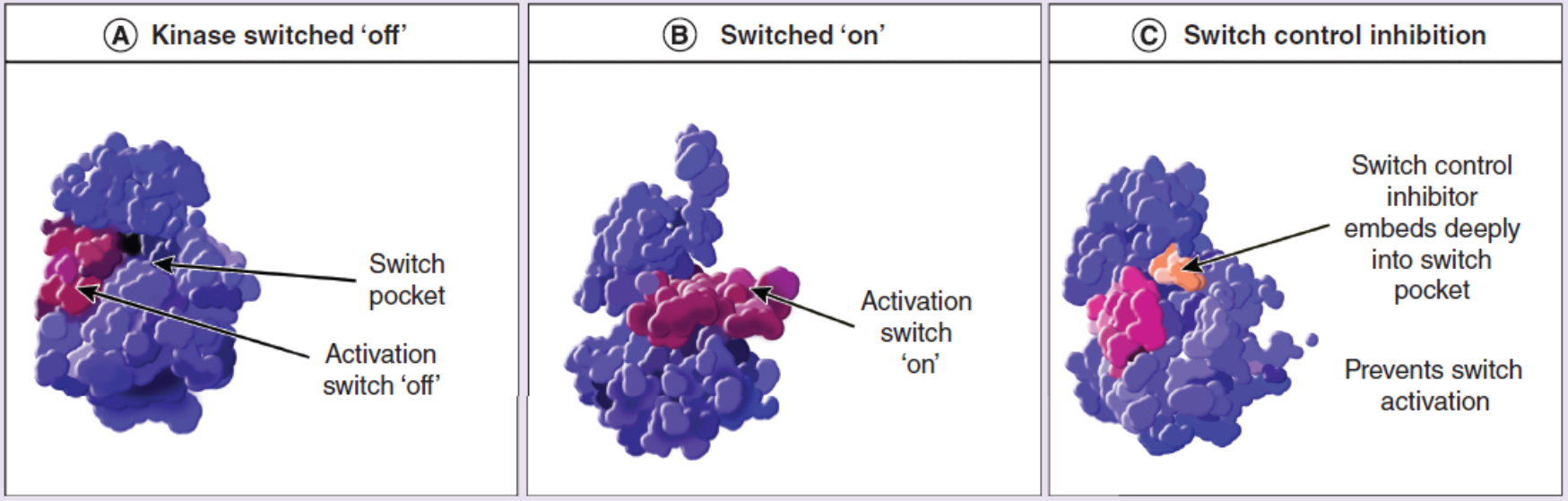
“Bespoke” TKI drugs for GIST

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.

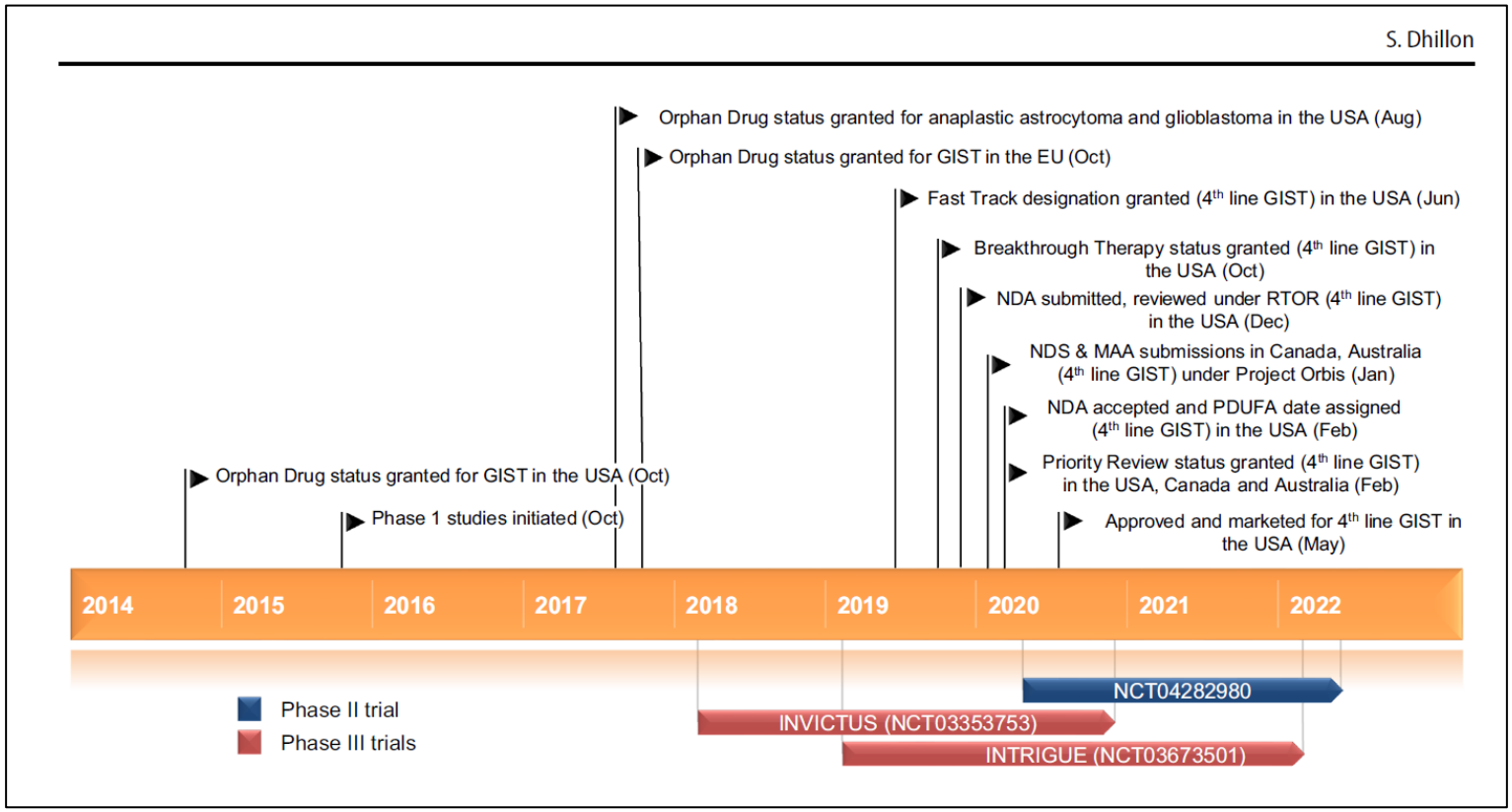
Ripretinib is a "switch-pocket" inhibitor; binds to KIT and prevents the protein from switching into its "active" conformation.



Nemunaitis *et al.*, 2020

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

Drilon, A., TRK inhibitors in TRK fusion-positive cancers, *Ann. Oncol.* 2019.

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

Banks *et al.*, Discovery and pharmacological characterization of AZD3229, a potent KIT/PDGFRα inhibitor for treatment of gastrointestinal stromal tumors, *Sci. Transl. Med.*, 2020.

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

david.josephy@liferaftgroup.ca