Life Fest, New Orleans
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An Introduction to the Biology and Molecular Biology of GIST

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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?
- What are the “ICC” cells where GISTs start ?
- What are KIT and PDGFRA?
- What are IHC and mutational testing?
- How does Gleevec (imatinib) work?

What causes GIST?

Most* GISTs occur "sporadically", as a result of a random mutation; such mutations are not inherited and are not passed on to one's children.

No environmental, occupational, dietary, or lifestyle causes of GIST are known – and if there were any major risk factors, they would have been identified by now!

***A few cases of familial (germline) GIST are known, but this is very rare.**

Cancers and cells:

Cancers can begin in almost any type of cell in the body.

To determine the best treatment of a cancer, we need to know the type of cell from which it developed.

The cell type (*not the organ*) defines a cancer.

→ *Basal cell carcinoma* and *melanoma* are both "skin cancers" but they are completely different diseases.

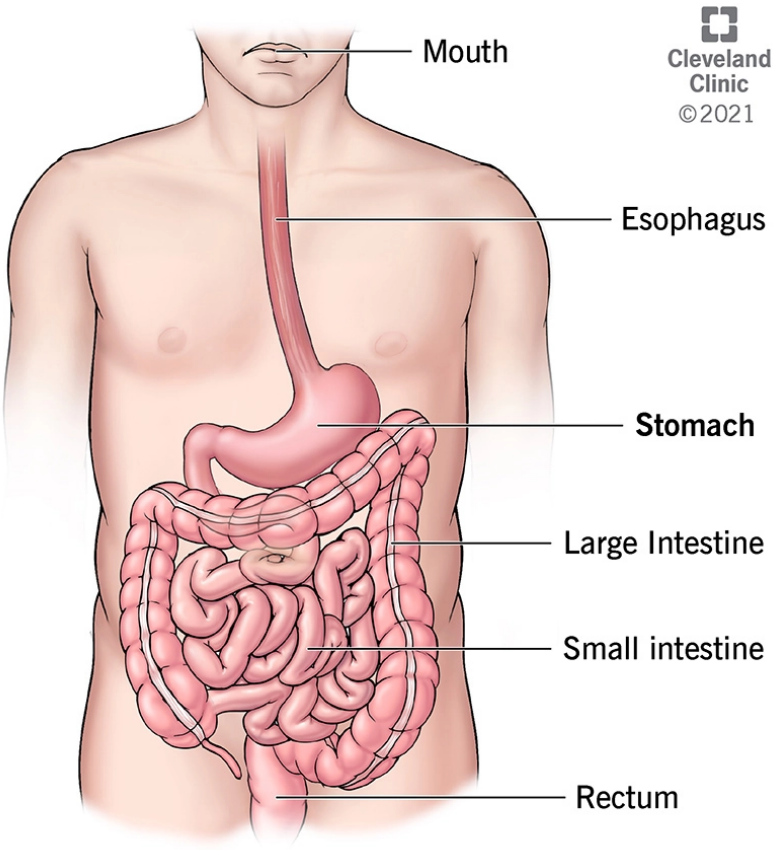
Carcinomas *vs.* sarcomas

***Carcinomas** are cancers that arise in **epithelial** tissues: the skin, or the tissues that line the organs: these are the common cancers of the breast, colon, prostate, lung, stomach, etc.*

***Sarcomas** are cancers that arise in **connective/ supportive** tissues. Examples: osteosarcoma (bone); liposarcoma (fat); angiosarcoma (blood vessels).*

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

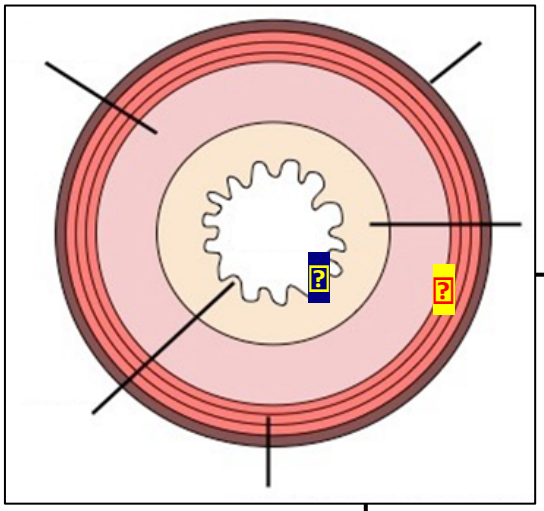
GIST is a sarcoma of the gastrointestinal tract.



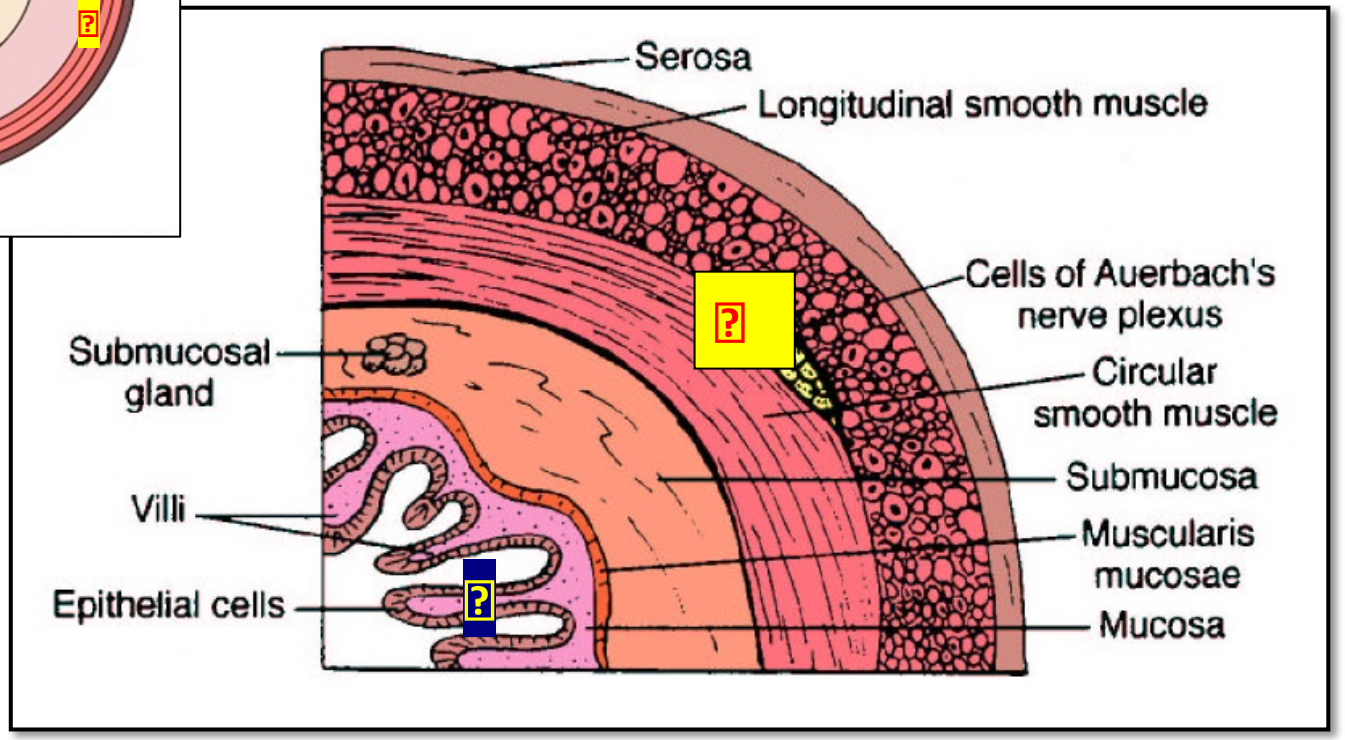
GI carcinomas (stomach and colon) are common.

GIST (GI sarcoma) is rare.

Cross-section of the GI tract

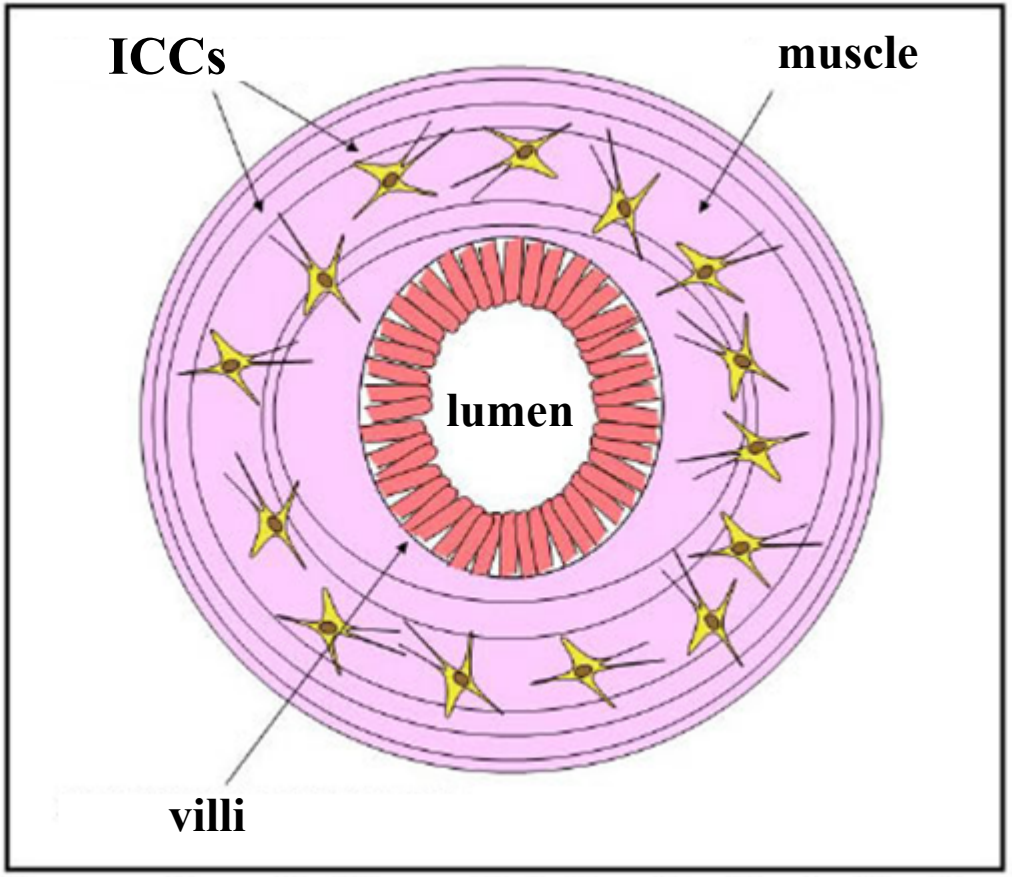


- carcinomas start in the epithelial lining (the body's "outside" surface)



- GISTs (sarcomas) start in the muscular wall

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

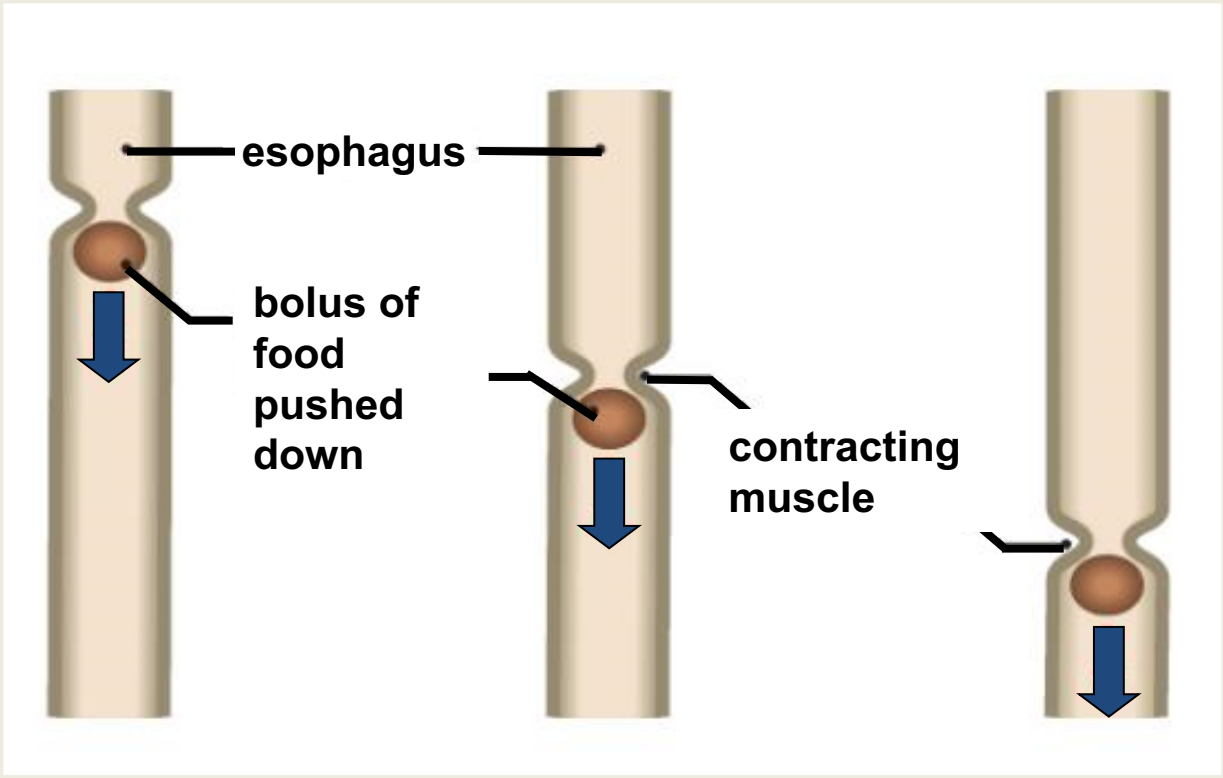


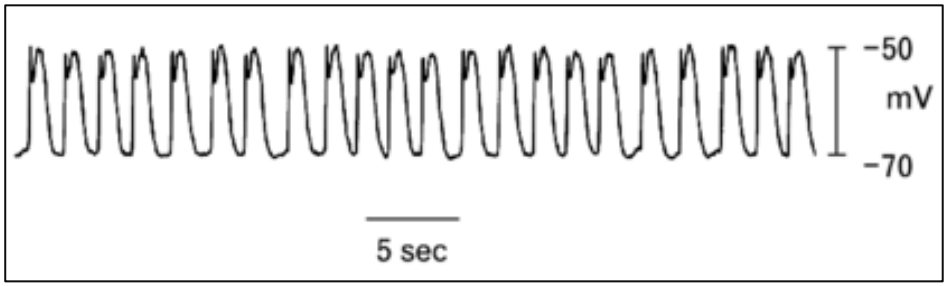
Cajal (1852-1934)

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)

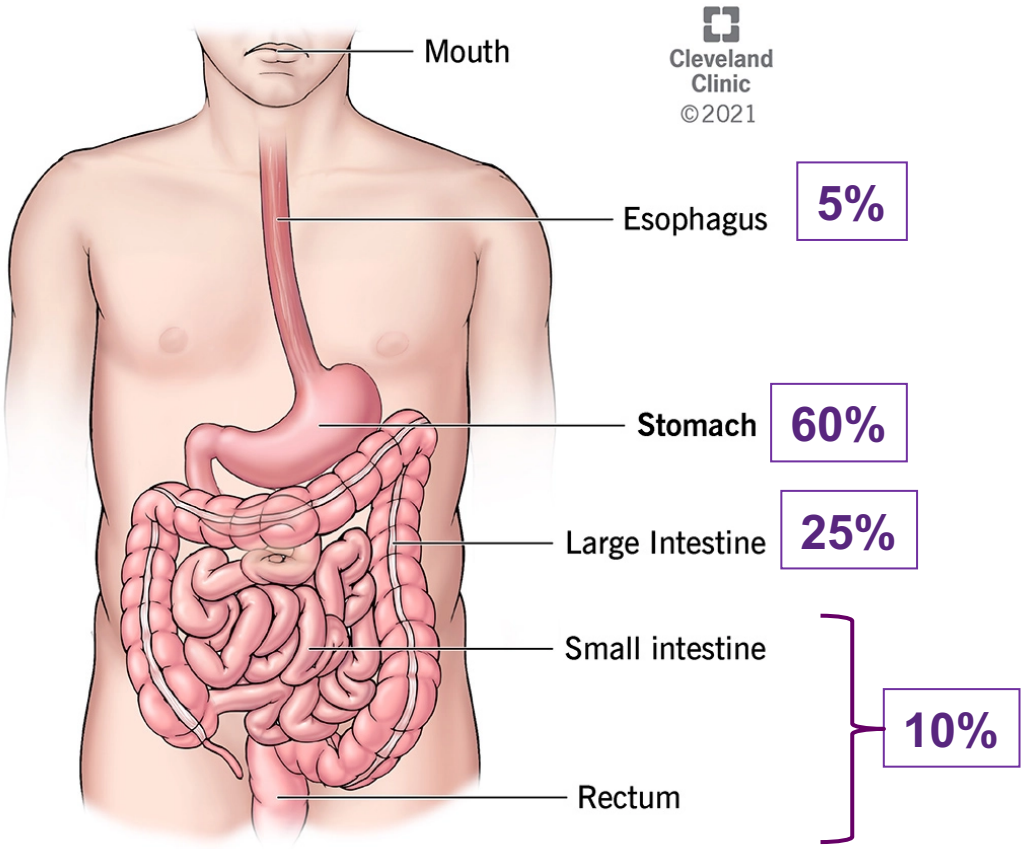
Peristalsis **- the coordinated waves of muscle action that push food through the GI tract; ICCs send out the regular electrical pulses that stimulate the GI muscles to contract.**





ICCs: pacemaker activity (mouse)

GIST tumors arise in the same cell type (ICC), regardless of their location along the GI tract.



Nevertheless, there are some differences in biology and prognosis between GISTs at different sites.

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).

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.

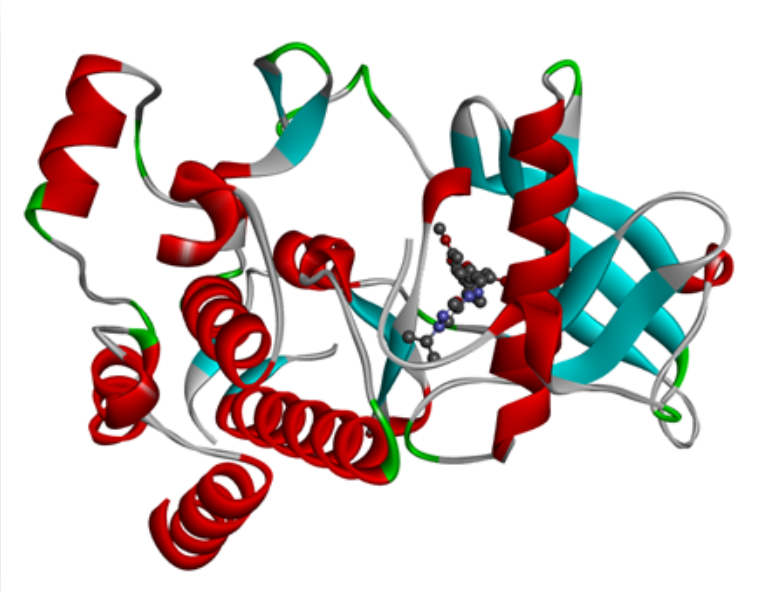
A localized GIST *may be cured* by surgery; but, even after a successful surgery, GIST may recur.

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

Systemic (drug) therapy is needed.

Molecular Biology of GIST

Proteins and Genes



Proteins and Genes

Proteins are the cell's engineers, performing the essential biochemical and control functions.

Genes (DNA) are the codes ("blueprints") for proteins.

The human genome encodes >30,000 different proteins.

Proteins and Genes

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

A = alanine	C = cysteine	D = aspartic acid
H = histidine	K = lysine	*etc.*

Lengths of protein amino acid sequences: anywhere from a few dozen to tens of thousands.

Genes (DNA) are the codes ("blueprints") for proteins.

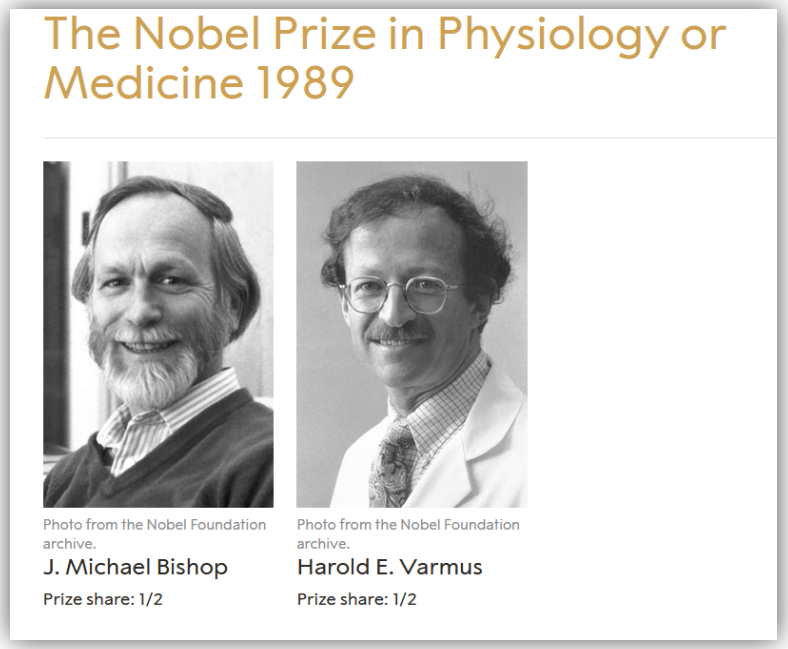
What is a mutation?

- A change in the DNA sequence encoding a protein.

- Mutations occur randomly, but cells carrying certain mutations will die, while others will grow faster.

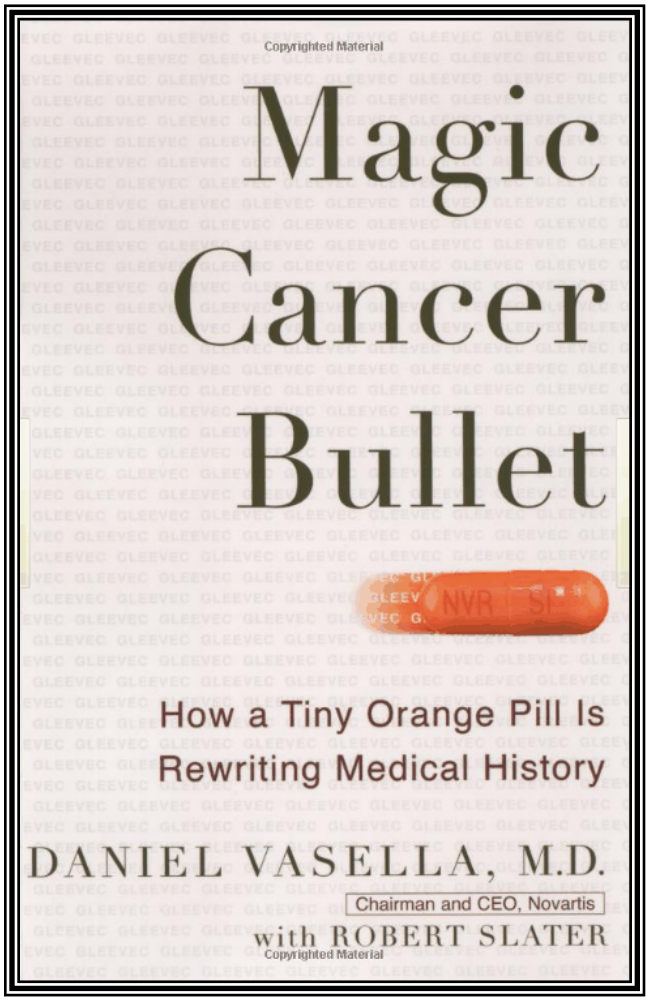
Oncogenes

The concept of targeted cancer chemotherapy grew out of the discovery of *oncogenes*: genes which, when mutated, produce proteins that drive uncontrolled cell proliferation.



Imatinib was one of the first drugs targeting the protein product of an oncogene; imatinib was first used for treatment of CML (a type of leukemia) in 1998, with revolutionary success.


MAY 28, 2001
www.time.com AOL Keyword: TIME
TIME
THERE IS NEW AMMUNITION IN THE WAR AGAINST CANCER.
THESE ARE THE BULLETS.
Revolutionary new pills like GLEEVEC combat cancer by targeting only the diseased cells. Is this the breakthrough we've been waiting for?
May 28, 2001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How a Tiny Orange Pill Is Rewriting Medical History
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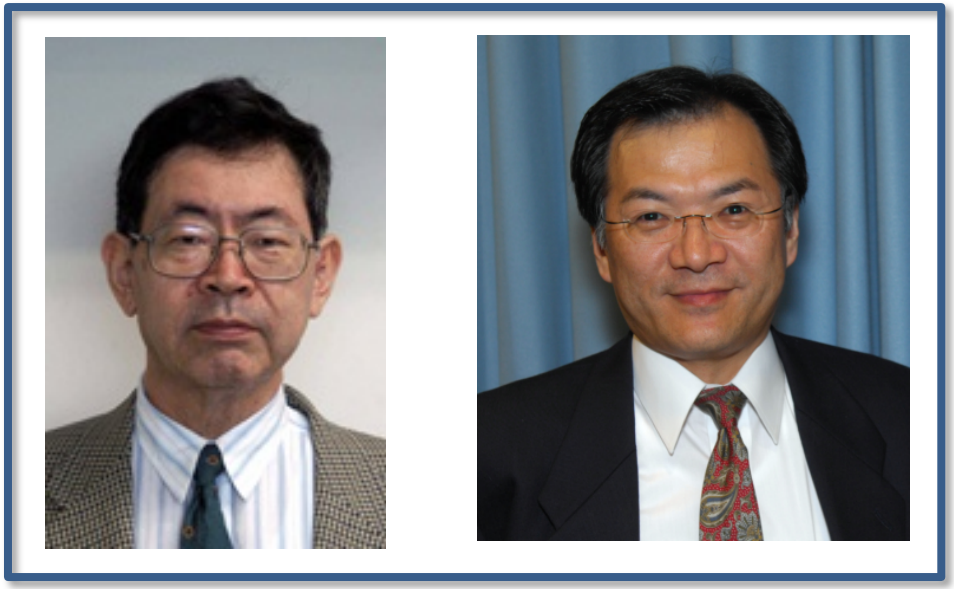
GIST and KIT: the 1998 breakthrough that revolutionized GIST diagnosis and treatment.

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

  (very few other cells in the body do so)

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



Yukihiko Kitamura, M.D. Seiichi Hirota, M.D.

Osaka University Medical School

"Targeted" drugs for treating CML and GIST

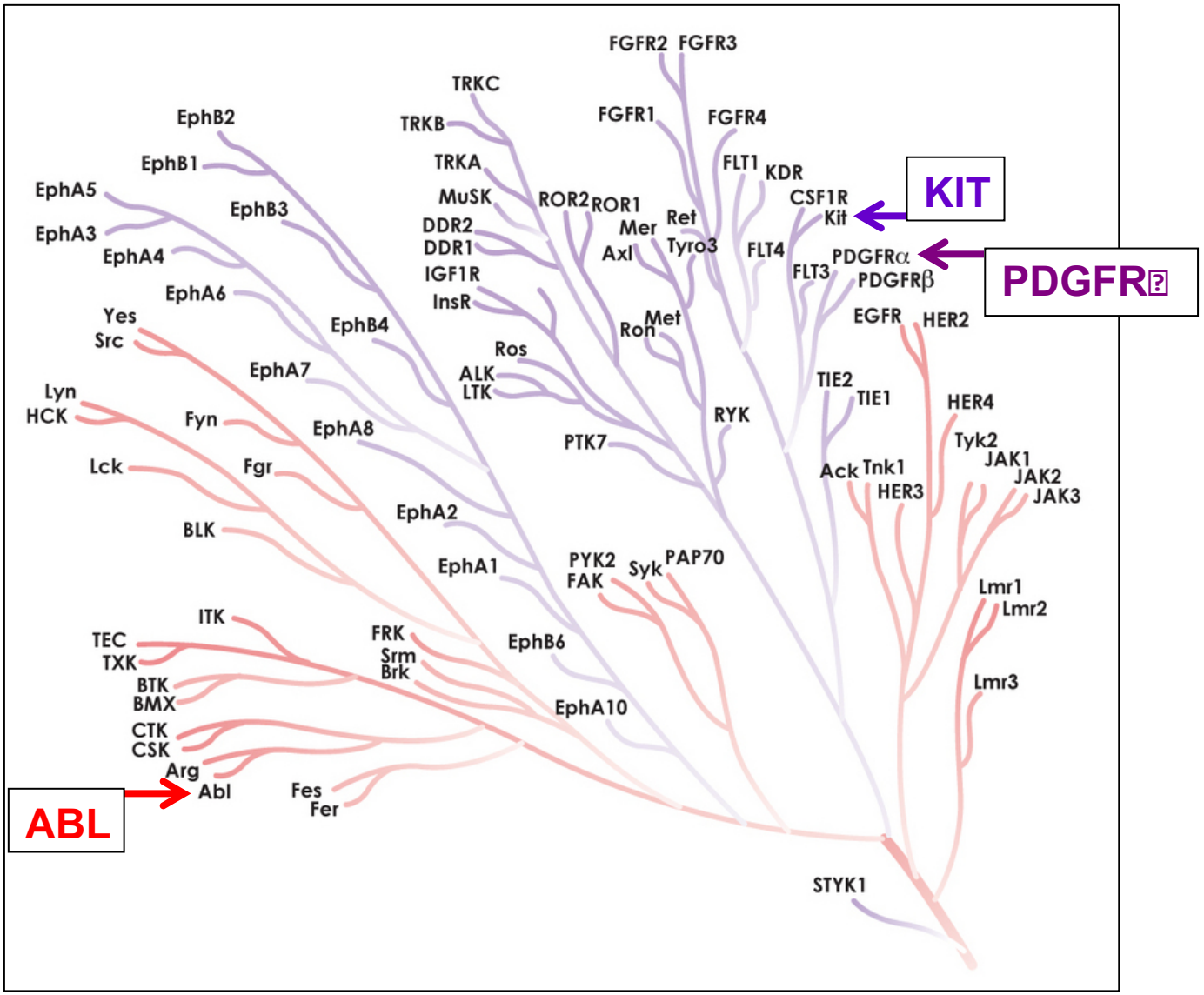
Chronic Myelogenous Leukemia (CML) is a rare leukemia (cancer of the blood). CML seems completely different from GIST … but the two diseases turned out to be related, at the molecular level.

The mutation causing CML is in a gene called "*ABL*"; ABL and KIT proteins are "cousins", with similar structures.

ABL and KIT proteins are "tyrosine kinase" enzymes. Drugs that inhibit (shut down) those enzymes are "tyrosine kinase inhibitors" (TKIs).

KIT is one member of a large family of proteins. PDGFR□ is a “sister”; ABL is a “cousin”.

human tyrosine kinases

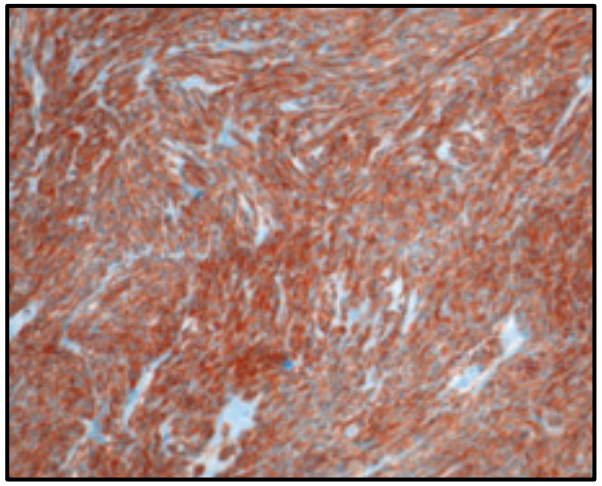


KIT ("c-Kit" or "CD117")

The KIT protein is made (expressed) by only a few types of adult cells, including the Interstitial Cells of Cajal … 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* a slice of the tissue sample (obtained at surgery) with an *antibody* that recognizes KIT protein. A pathologist examines the stained tissue under the microscope.



Di Vizio *et al.*, 2008

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

Immunohistochemistry (IHC) *vs.* Mutational testing: different tests, different questions, different answers

	Immunohistochemistry	Mutational testing
What it is:	**Staining for the KIT protein**	**DNA sequencing of the *KIT* gene**
What it tests for:	**expression of KIT protein by the tumour cells**	**mutations in the *KIT* gene in the tumour cell DNA**
What it tells us:	**is the tumour GIST? (often, this simply confirms the diagnosis)**	**is the tumour a *KIT*-mutant GIST (and, if so, what is the *KIT* mutation?)***
What it requires:	**a tumour sample (biopsy or surgery)**	**a tumour sample (*e.g.*, FFPE: Formalin-Fixed Paraffin-Embedded)**
Will the test be performed by the pathology lab?	***always***	***sometimes*; LRG strongly recommends that patients push to have mutational testing done!**

*If no mutation is found in the *KIT* gene, the lab will probably go on to examine other genes, *e.g. PDGFR*, *RAS*, *BRAF* …

The GIST-KIT connection (2022 update)

We now realize that there are many types of GIST, differing at the genetic level.

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

Of the GISTs that do not have KIT mutations:

- About 15% have a mutation in a related gene, *PDGFR*.

- Some 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 types of GIST express KIT protein – whether or not the *KIT* gene is mutated.**

***almost* all, anyway; there are rare exceptions.**

Development of our understanding of mutations in GIST

1998 *KIT* mutations (~75%; imatinib and other TKIs)
... the remaining 25% were called "wild-type GIST" (??)

2003 *PDGFRA* mutations (avapritinib, 2020)

2006 Germline *NF1* mutations

2007 Germline *SDH* mutations (not yet druggable)

2008 *BRAF* V600E mutation (vemurafenib)

2014 *SDHC* hypermethylation (epigenetic)

2016 *NTRK* fusions (larotrectinib)
FGFR1 fusions

Protein structure: Exons

Many proteins consist of several 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 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
```

The KIT protein: 21 exons

1 [1]**MRGARGAWDF LCVLLLLLRV QT**[2]GSSQPSVS PGEPSPPSIH PGKSDLIVRV GDEIRLLCTD

61 PGFVKWTFEI LDETNENKQN EWITEKAEAT NTGKYTCTNK HGLSNSIYVF VR[3]**DPAKLFLV**

121 **DRSLYGKEDN DTLVRCPLTD PEVTNYSLKG CQGKPLPKDL RFIPDPKAGI MIKSVKRAYH**

181 **RLCLHCSVDQ EGKSVLSEKF ILKVRP**[4]AFKA VPVVSVSKAS YLLREGEEFT VTCTIKDVSS

241 SVYSTWKREN SQ[5]**TKLQEKYN SWHHGDFNYE RQATLTISSA RVNDSGVFMC YANNTFGSAN**

301 **VTTTLEVV**[6]DK GFINIFPMIN TTVFVNDGEN VDLIVEYEAF PKPEHQQWIY MNRTFTDKWE

361 DYPKSENESN I[7]**RYVSELHLT RLKGTEGGTY TFLVSNSDVN AAIAFNVYVN** [8]TKPEILTYDR

421 LVNGMLQCVA AGFPEPTIDW YFCPGTEQ[9]**RC SASVLPVDVQ TLNSSGPPFG KLVVQSSIDS**

481 **SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNK**[10]EQIHPHT LFTPLLIGFV IVAGMMCIIV

541 MILTYKYLQ[11]**K PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS F**[12]GKTLGAGAF

601 GKVVEATAYG LIKSDAAMTV AVKMLK[13]**PSAH LTEREALMSE LKVLSYLGNH MNIVNLLGAC**

661 **TIG**[14]GPTLVIT EYCCYGDLLN FLRRKRDSFI CSKQEDHAEA ALYKNLLHSK ESS[15]**CSDSTNE**

721 **YMDMKPGVSY VVPTKADKRR SVRI**[16]GSYIER DVTPAIMEDD ELALDLEDLL SFSYQVAKGM

781 AFLASKN[17]**CIH RDLAARNILL THGRITKICD FGLARDIKND SNYVVKGN**[18]AR LPVKWMAPES

841 IFNCVYTFES DVWSYGIFLW ELFSL[19]**GSSPY PGMPVDSKFY KMIKEGFRML SPEHAPAE**[20]MY

901 DIMKTCWDAD PLKRPTFKQI VQLIEKQISE STN[21]**HIYSNLA NCSPNRQKPV VDHSVRINSV**

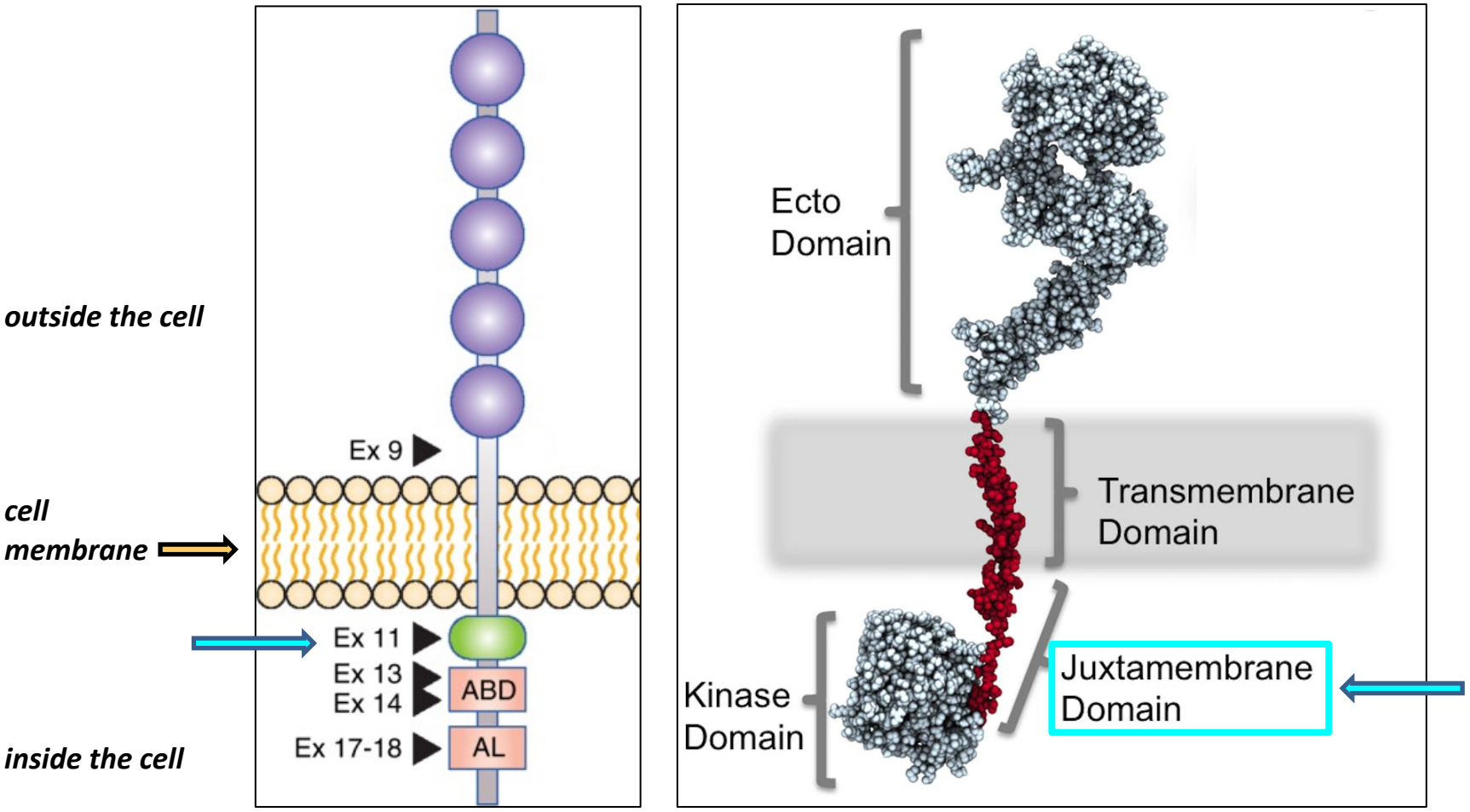
961 **GSTASSSQPL LVHDDV**

The KIT protein: exons 9 and 11

```
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 RLKGTEGC[exon 9]LVSNSDVN AAIAFNVYVN TKPEILTYDR
421  LVNGMLQCVA AGFPEPTIDW YFCPGTEQRC SASVLPVDVQ TLNSSGPPFG KLVVQSSIDS
481  SAFKHNGTVE CKAYNDVGKT SAYFNFAFKG NNKEQIHPHT LFTPLLIGFV IVAGMMCIIV
541  MILTYKYLQK PMYEVQWKVV EEINGNNYVY IDPTQLPYDH KWEFPRNRLS FGKTLGAGAF
601  GKVVEATAY[exon 11]DAAMTV 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 the KIT protein to "switch" from its "inactive" form to its "active" form, signaling the GIST cell to grow and divide.



Two "cartoon" representations of the structure of the KIT protein.
ABD = ATP-binding domain; AL = activation loop

KIT and PDGFRA

The KIT and PDGFRA proteins are enzymes – "tyrosine kinases" – that acts on other proteins, modulating their activities (triggering a "signal transduction cascade").

The *KIT* and PDGFRA genes are "oncogenes".

An oncogene is a gene which, when mutated, encodes a protein product that instructs a cell to keep dividing: a "stuck gas pedal".

In about 90% of GIST cases – *but not 100%* – either the *KIT* gene or the *PDGFRA* gene (*but not both*) is mutated; consequently, an aberrant form of KIT or PDGFRA protein is produced by the GIST tumour cells, "driving" the cells to proliferate.

KIT mutations in GIST are *somatic* mutations.

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)**
- **carried by the tumor cells, but not passed on to a patient's children**

Diversity of mutations in GISTs

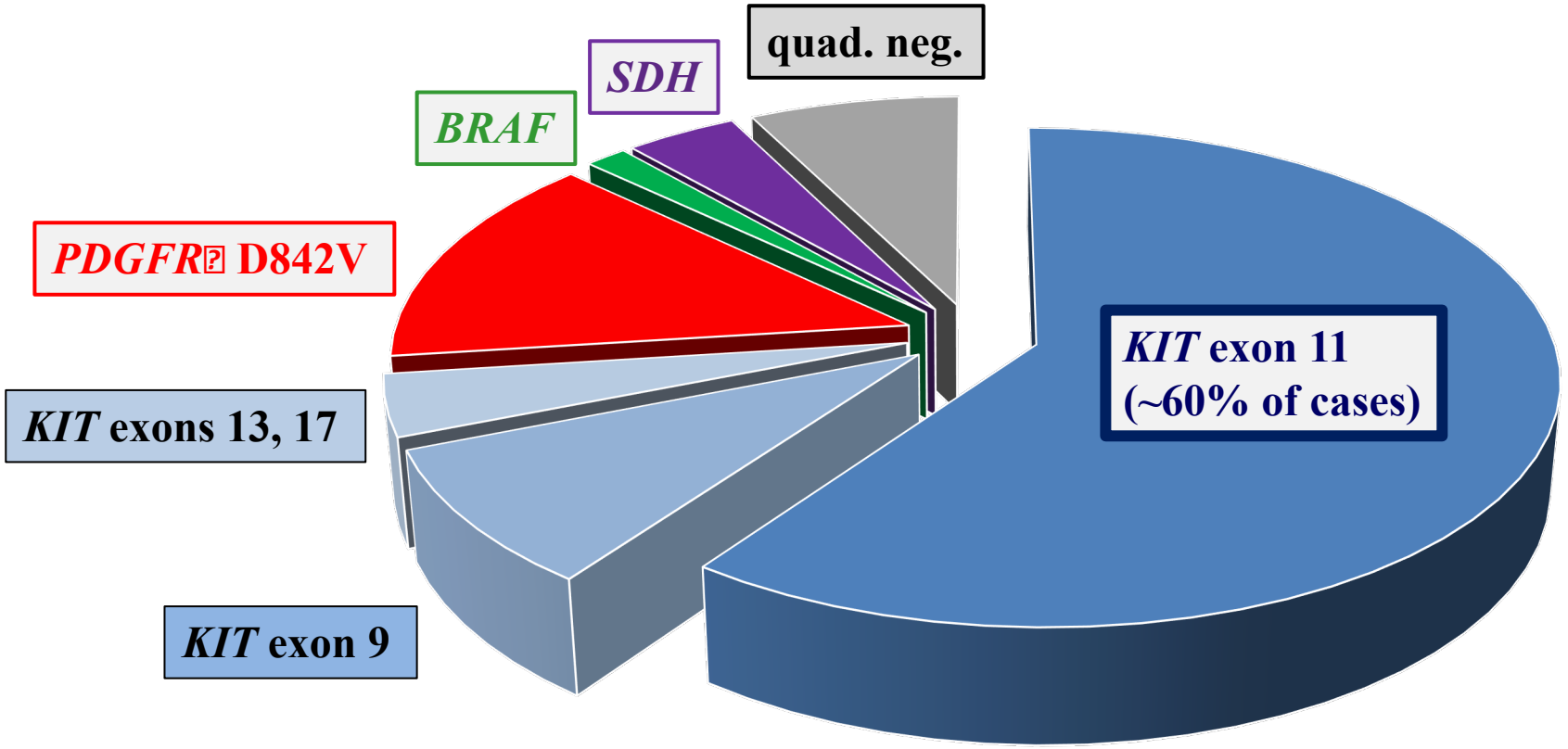
GIST "driver" mutations can occur at many different sites in the *KIT* gene, affecting many different sites in the KIT protein … and sometimes GIST driver mutations occur in genes other than *KIT*: *PDGFR*, *SDH*, *BRAF*, *NTRK*, etc.

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).

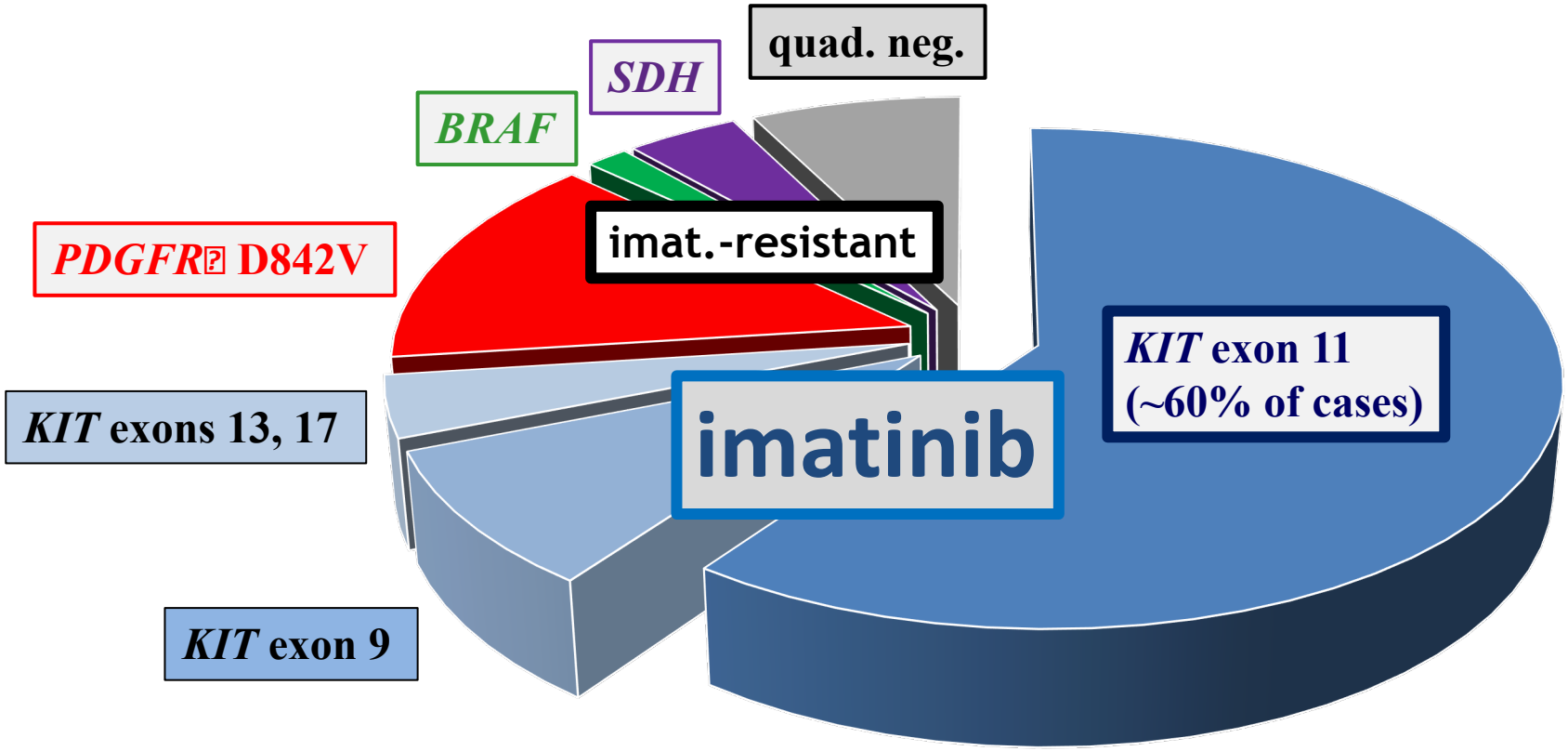
(Baveno declaration, 2008).

KIT and PGFRA mutations drive *most* GISTs.



Approximate distribution of "driver" mutations in GISTs

Approximate distribution of "driver" mutations in GISTs



Avapritinib (BLU-285) for treatment of PDGFRA D842V GIST

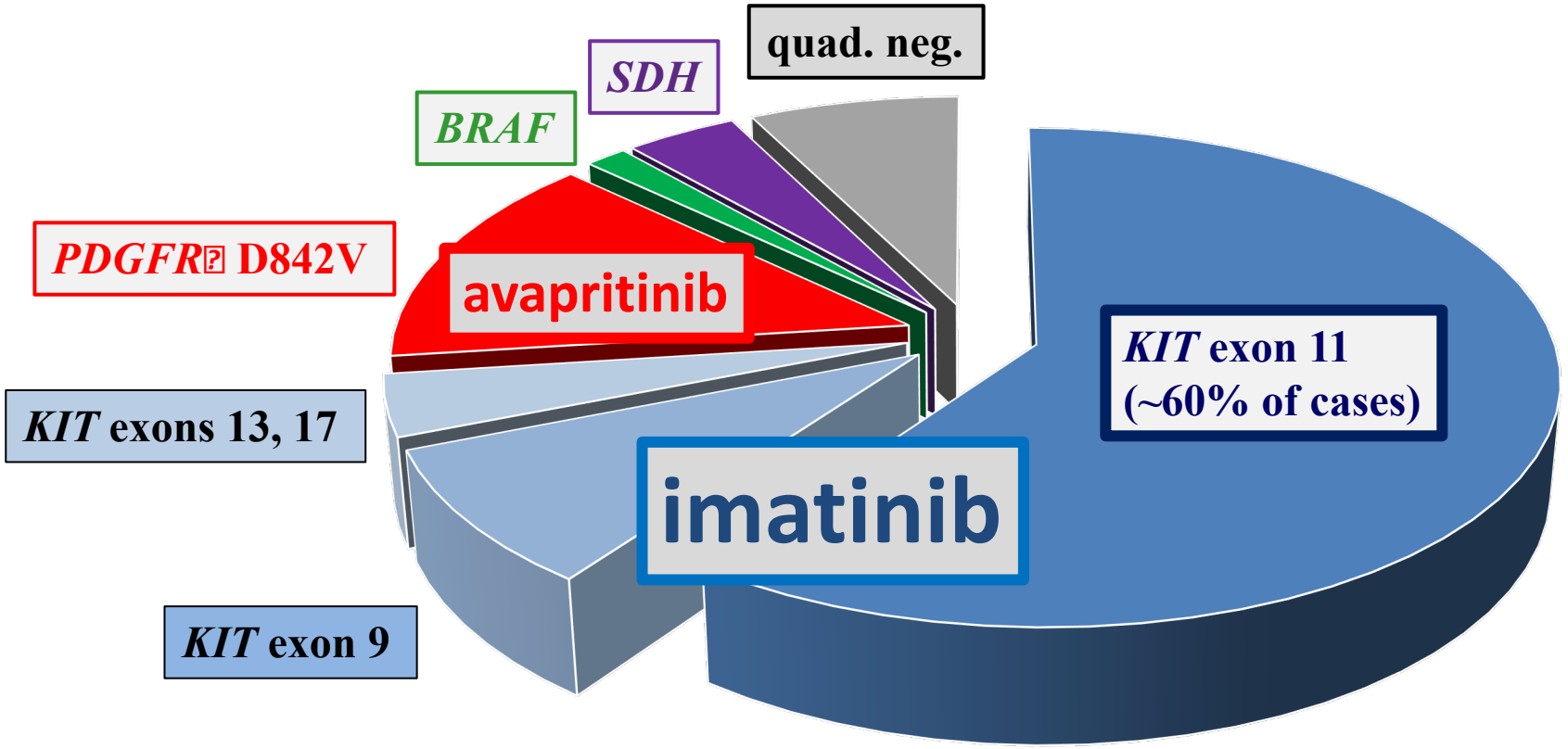
Smrke *et al.*, Avapritinib in the treatment of PDGFRA exon 18 mutated GIST, *Future Oncol.* 16:1639-1646, 2020.

"GIST patients with *PDGFRA* mutations are an important subgroup that commonly arise in the stomach and are associated with a more indolent disease course.

Importantly, the most common PDGFRA molecular subtype, the D842V mutation in exon 18 … is imatinib insensitive [and] poor responses to imatinib have been seen clinically.

Avapritinib (BLU-285) … has shown >90% response rates in patients with PDGFRA exon 18 D842V-mutated GIST. … This drug should be the standard of care for patients with PDGFRA exon 18 D842V-mutated GIST."

Approximate distribution of "driver" mutations in GISTs



The need for universal mutational testing of GISTs is now undeniable!

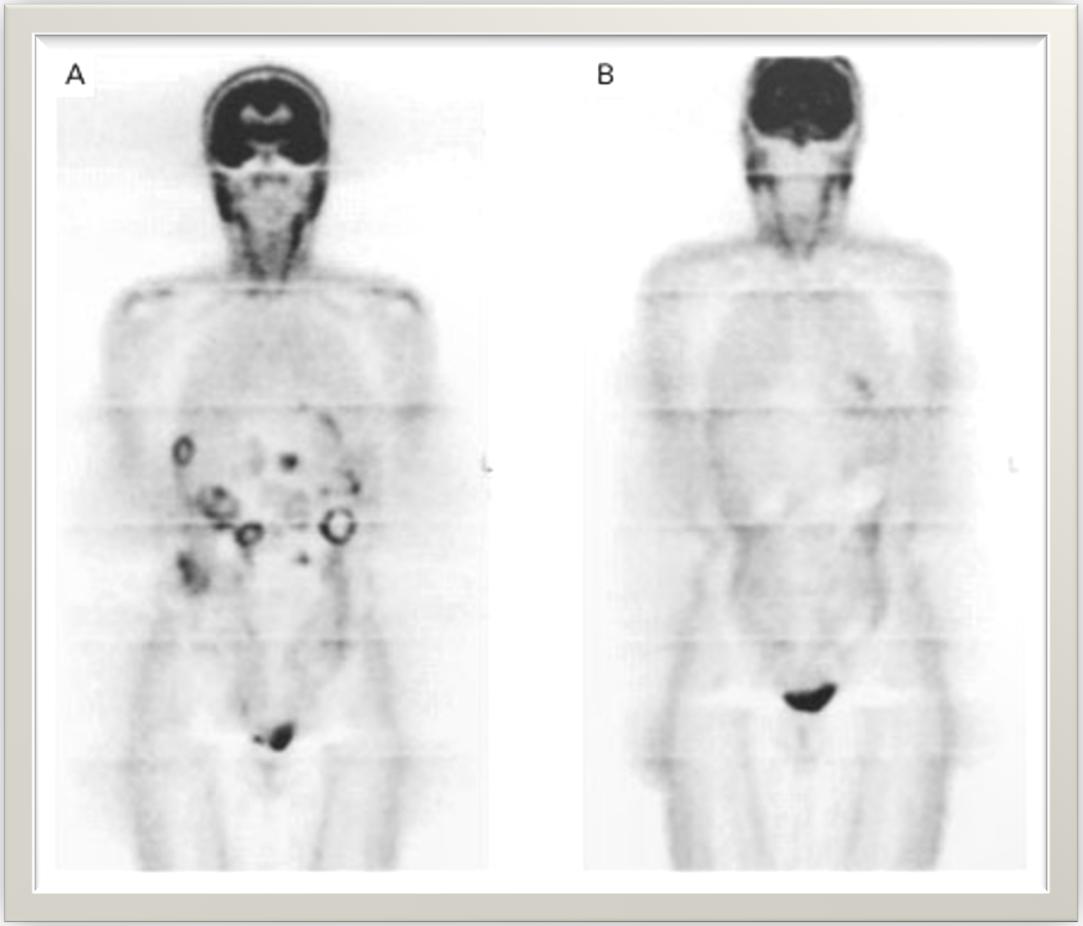
In the early years of the imatinib era, a doctor could still argue, *"Imatinib is the only drug I have, as first-line treatment for GIST. So, why should I ask for mutational testing of a GIST patient? The result won't change my treatment plan! If imatinib isn't working, I will just tell the patient to stop taking it."*

Once we knew that *PDGFRA* GISTs do not respond to imatinib, this argument no longer made any sense. *Why would one prescribe an expensive drug (with sometimes-serious side effects), knowing that it is not going to work?*

Now, with effective drugs such as avapritinib and larotrectinib available as first-line therapies for sub-classes of GIST, the argument is untenable. We need universal* mutational testing of GISTs.

*with the possible exception of small, localized "wait-and-see" GISTs

The development of targeted drugs for treating GIST



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

The first three TKIs approved for use in GIST:

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

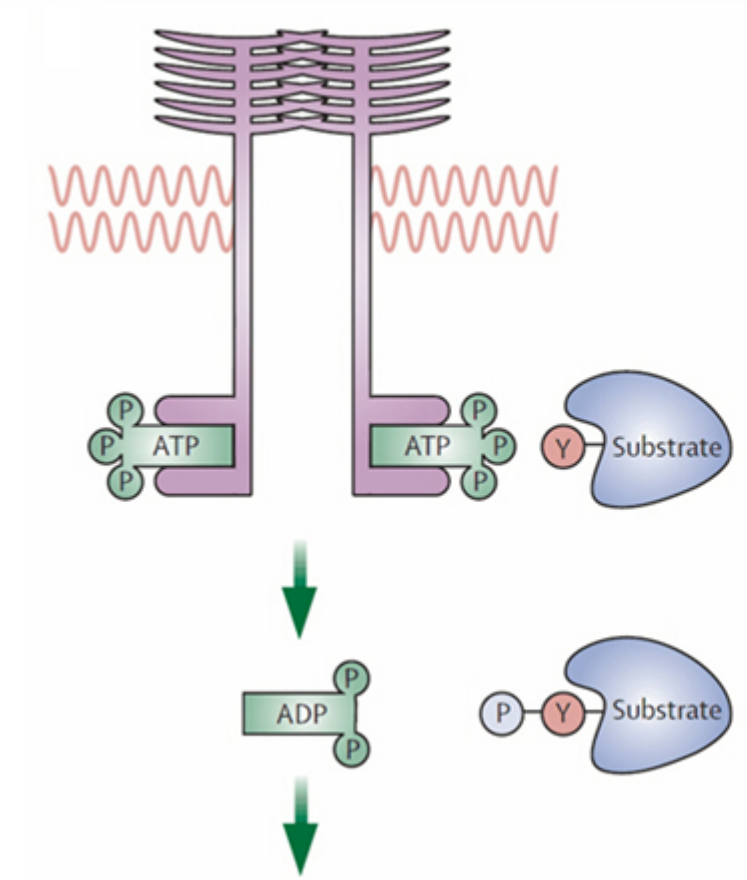
Second-line: Sunitinib (Sutent - Pfizer; 2006)

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

(The 'ib" ending indicates an enzyme inhibitor)

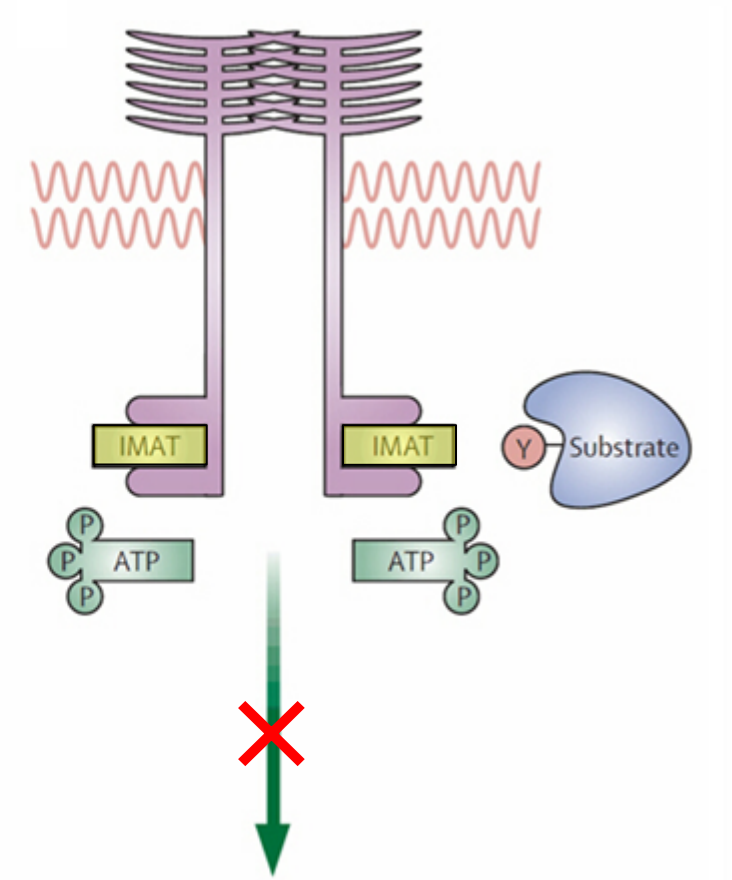
All of these drugs act by the same mechanism - blocking the binding of ATP (cellular fuel) to KIT.

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.**
- **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.**

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