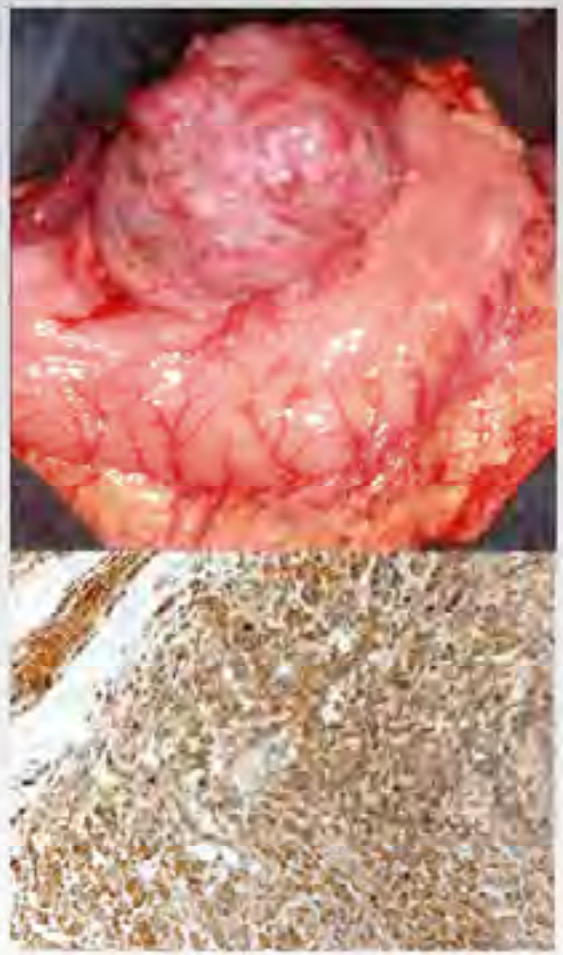
IDENTIFICAZIONE di ALTERAZIONI GENICHE
e
NUOVE PROSPETTIVE TERAPEUTICHE
dei GIST

A.M.PISACANE
IRCC Candiolo

Gastrointestinal Stromal Tumors (GIST)



- Most frequent sarcoma of the GI tract
- Incidence: Approximately 1.5 new cases per 100,000 each year[a]
- Diagnosis by histology and immunohistochemistry of KIT and CD34[a]
- Molecular biology techniques (eg, mutational analysis) used to identify subtypes of disease

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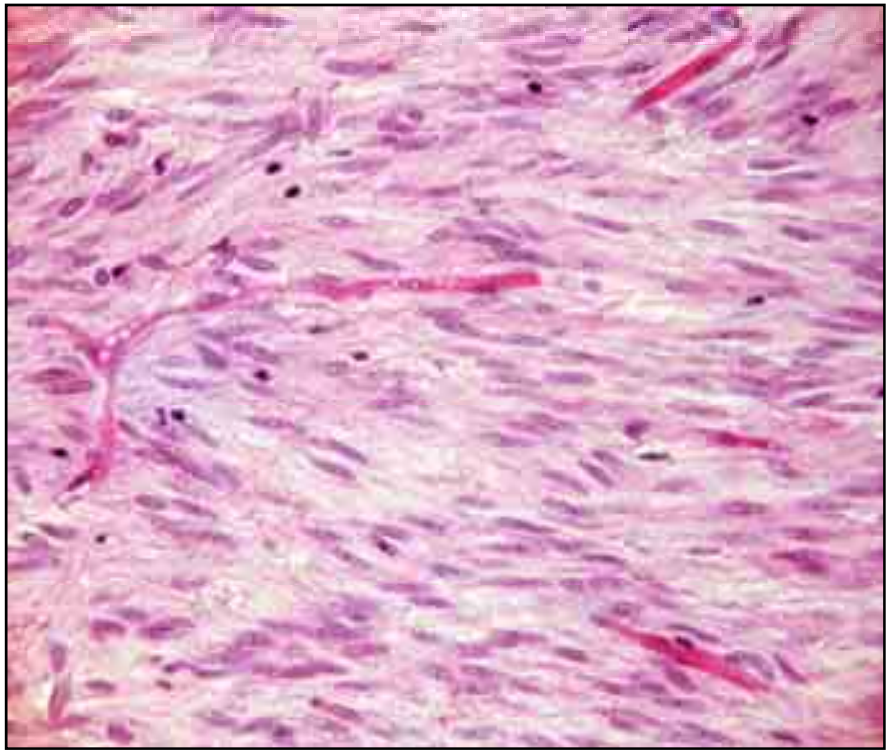
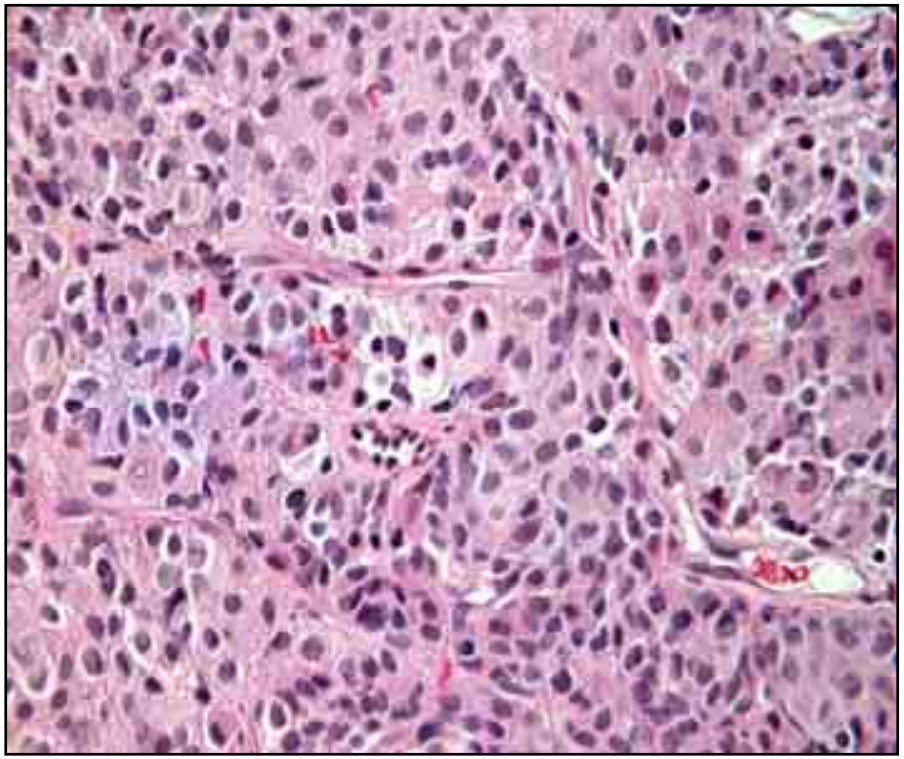
a. Casali PG, Blay J-Y. *Ann Oncol.* 2010;21(Suppl 5):v98-102.

GIST: Major Morphologic Patterns

Spindle Cell (70%)



Epithelioid (9%)



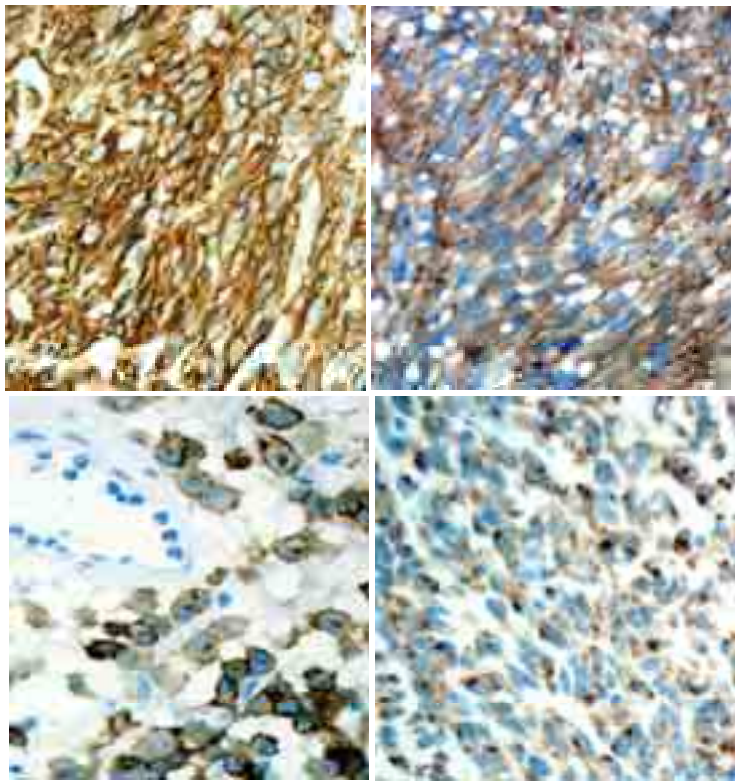
Other-> mixed 21%

Courtesy of Dr. C. Corless.

Immunohistochemistry

- ~95% of reported cases of GIST are positive for KIT **(CD117)**

- Other markers often positive in GIST
  - **CD34** (mesenchymal/hematopoietic precursor cell marker)
    - **Positive in 60%-70%**
  - **Smooth-muscle actin**
    - **Positive in 15%-60%**
  - **S-100**
    - **Positive in 10%**



Different KIT staining patterns in GIST

Courtesy of Dr. C. Corless.

Miettinen and Lasota. *Virchows Arch*. 2001;438:1.

DOG1 Antibody in the Differential Diagnosis of Gastrointestinal Stromal Tumors
A Study of 1840 Cases

The overall sensitivity of DOG1 and KIT in GISTs was nearly identical: 94.4% and 94.7%

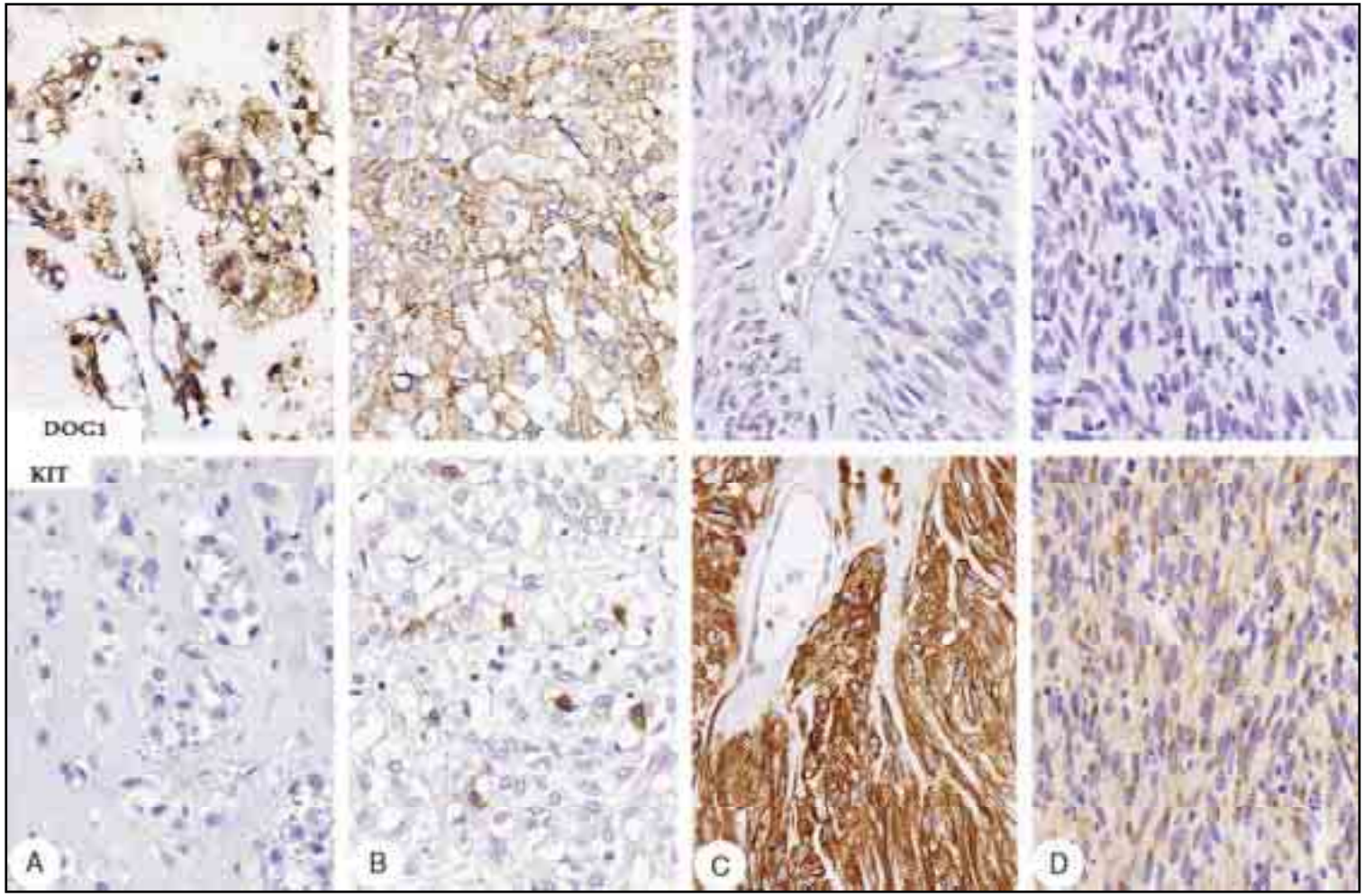
Negativity for both DOG1 and KIT was observed in 2.6% of GISTs of GI tract.

Approximately half of KIT-negative GISTs were positive for DOG1, and just half of DOG1-negative GISTs were KIT-positive.

These results indicate that use of both markers together is beneficial in problem cases, such as tumors with unexpectedly KIT-negative or positive results.

Miettinen M. et al: Am J Surg Pathol 2009;33

DOG 1



DOG1 Antibody in the Differential Diagnosis of Gastrointestinal Stromal Tumors: A Study of 1840 Cases.
Miettinen, Markku; Wang, Zeng-Feng; Lasota, Jerzy

American Journal of Surgical Pathology. 33(9):1401-1408, September 2009.
DOI: 10.1097/PAS.0b013e3181a90e1a

Paired examples of DOG1 (upper row) and KIT immunostaining (lower row) in 4 GIST. A, Gastric sclerosing epithelioid GIST and another gastric epithelioid GIST are positive for DOG1 and negative for KIT. Note positive mast cells in lower panel of B. C, Gastric hypercellular spindle cell GIST negative for DOG1 and positive for KIT. D, Small intestinal GIST negative for DOG1 and positive for KIT. Note the perinuclear dot-like KIT immunostaining in this case.

GIST Risk Assessment: NIH/NCI Consensus Criteria

Risk for recurrence or metastasis	Tumor size	Mitotic count (per 50 HPF)
Very low	< 2 cm	< 5
Low	2-5 cm	< 5
Intermediate	≤ 5 cm	6-10
	6-10 cm	< 5
High	> 5 cm	> 5
	> 10 cm	Any
	Any	> 10

HPF = high-power fields

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Fletcher CDM, et al. *Hum Path*. 2002;33:459-465.

GIST Risk Assessment: AFIP Classification 2006

Risk category	Tumor size	No of mitosis /50 HPF	Tumor site	Risk for relapse (%)*
Very low	< 2 cm	≤ 5	All	0
Low	2-5 cm	≤ 5	All	1.9-4.3
	5-10 cm	≤ 5	stomach	3.6
	< 2 cm	> 5	Stomach	0†
Intermediate	> 10 cm	≤ 5	Stomach	12
	2-5 cm	> 5	Stomach	16
	5-10 cm	≤ 5	Intestine	24
High	> 10 cm	≤ 5	Intestine	52
	< 2 cm	> 5	Intestine	50†
	2-5 cm	> 5	Intestine	73
	5-10 cm	> 5	All	55-85
	> 10 cm	> 5	All	86-90

*Before imatinib era; †Insufficient statistical power, low number of patients

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Miettinen M, et al. *Arch Pathol Lab Med*. 2006;130:1466-1478.

Factors in Risk Stratification

- Mitosis count (NIH, AFIP, Gold)
- Tumor size (NIH, AFIP, Gold)
- Tumor site (modified NIH, AFIP)

Other important risk factors:

- Tumor rupture (> 80% risk for recurrence)
- Mutation status

Genetic Changes and Risk Stratification in GISTs

Gastrointestinal stromal tumor, uncommitted type, with monosomies 14 and 22 as the only chromosomal abnormalities Casorzo et al. *Cancer Genet Cytogenet. 1998 Apr 15;102(2*).
"Monosomies of chromosomes 14 and 22 are early events in the malignant transformation of the mesenchymal cell-originating gastrointestinal stromal tumors".

DNA sequence copy number changes in GI stromal tumors: tumor progression and prognostic significance. *El-Rifai. Cancer.Res.2000*
Several DNA copy number changes are related to the behavior of GISTs and can be used as prognostic markers for tumor progression.

Biological and clinical significance of cytogenetis abnormalities in low-risk and high-risk GI stomal tumors. *B.Gumawan. Hum. Pathol. 2002*
Loss of chromosome 14 and/or 22 is an early change in GIST tumorigenesis irrespective of site or differentiation, whereas malignant transformation and progression of GISTs appear to be associated with an increasing incidence of additional secondary aberrations.

In 1998, Seiichi Hirota and colleagues made the landmark discovery that the majority of GISTs harbor an activating mutation in the *KIT* oncogene.

KIT encodes the KIT receptor tyrosine kinase, which is the receptor for stem cell factor (SCF).

Binding of SCF to KIT induces KIT dimerization and activation

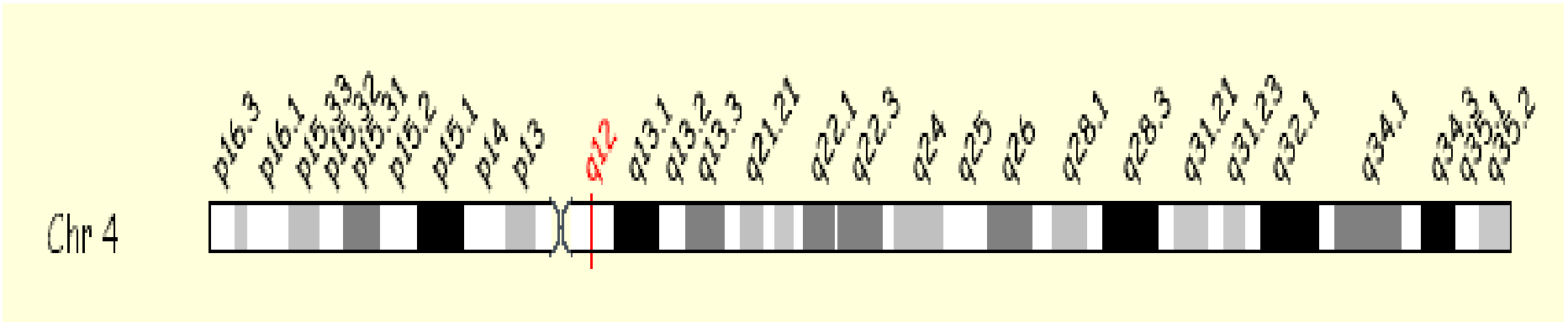
Brief Report

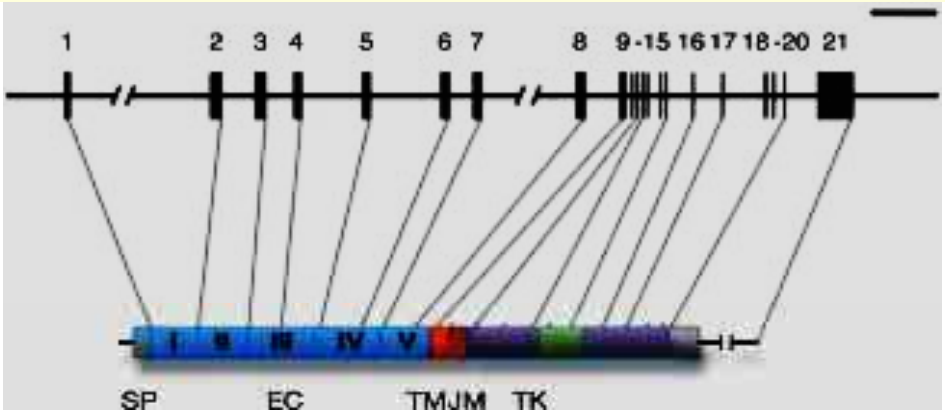
Effect of the Tyrosine Kinase Inhibitor STI571 in a Patient with a Metastatic Gastrointestinal Stromal Tumor

Heikki Joensuu, M.D., Peter J. Roberts, M.D.,
Maarit Sarlomo-Rikala, M.D.,
Leif C. Andersson, M.D., Pekka Tervahartiala, M.D.,
David Tuveson, M.D., Ph.D.,
Sandra L. Silberman, M.D., Ph.D.,
Renaud Capdeville, M.D., Sasa Dimitrijevic, Ph.D.,
Brian Druker, M.D., and George D. Demetri, M.D.

N Engl J Med, Vol. 344, No. 14 · April 5, 2001

…after three weeks of STI571 treatment, histologic examination of the liver metastasis showed myxoid degeneration and a few pyknotic cells, no staining for Ki-67, and only a few, scattered CD117-positive cells …





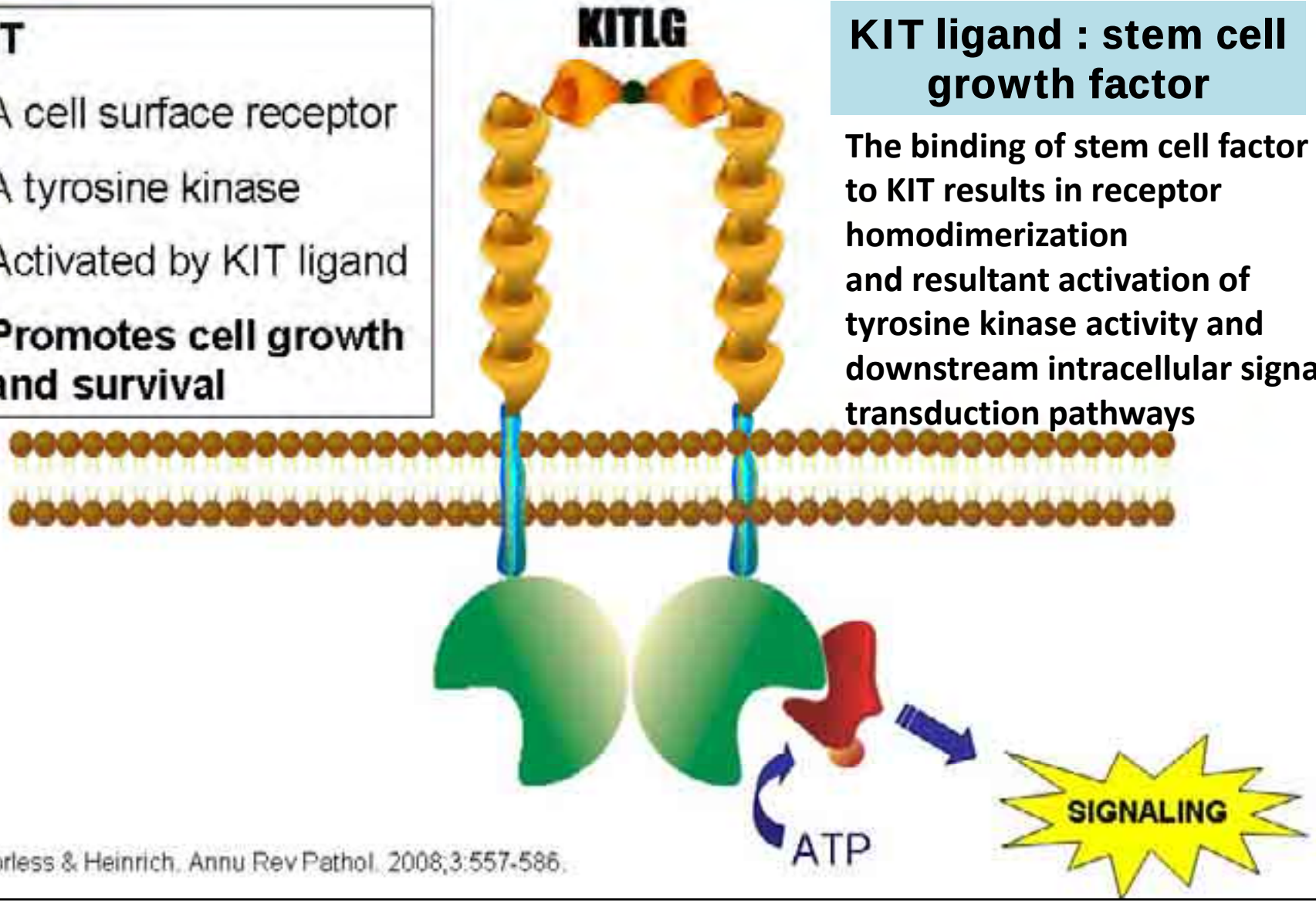
Genomic structure of the *KIT* gene in relation to the functional domains of KIT protein. SP, signal peptide sequence; EC, extracellular (Ig-like) domains I–V; TM, trans-membrane domain; JM, juxta-membrane domain; TK, tyrosine kinase domain.

Antonescu C. The Journal of Pathology 2010

KIT Signaling

KIT

- A cell surface receptor
- A tyrosine kinase
- Activated by KIT ligand
- **Promotes cell growth and survival**



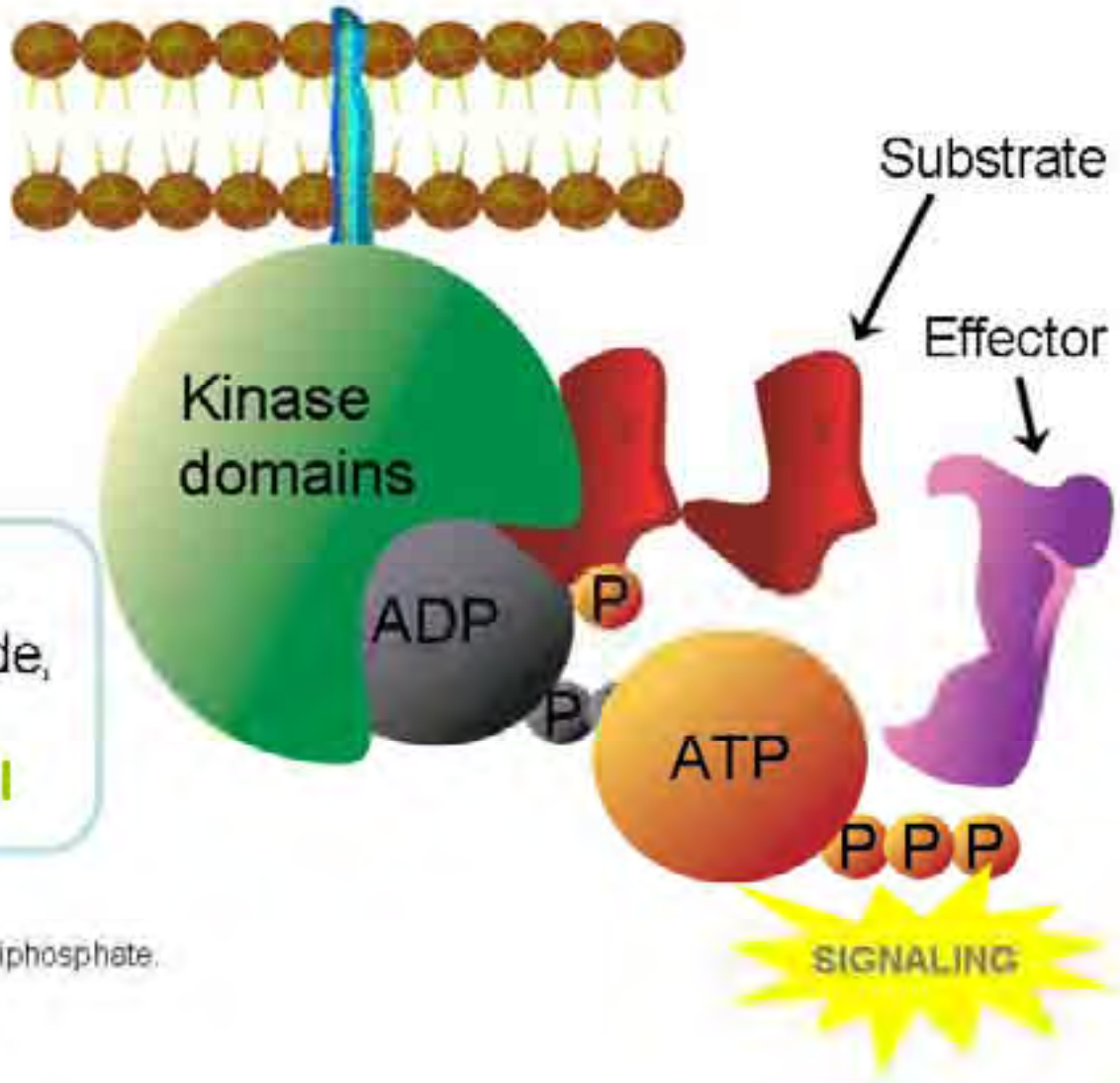
KIT ligand : stem cell growth factor

The binding of stem cell factor to KIT results in receptor homodimerization and resultant activation of tyrosine kinase activity and downstream intracellular signal transduction pathways

Corless & Heinrich. Annu Rev Pathol. 2008;3:557-586.

Normal KIT Signaling

A substrate protein (eg, PI3 kinase) is phosphorylated by KIT kinase



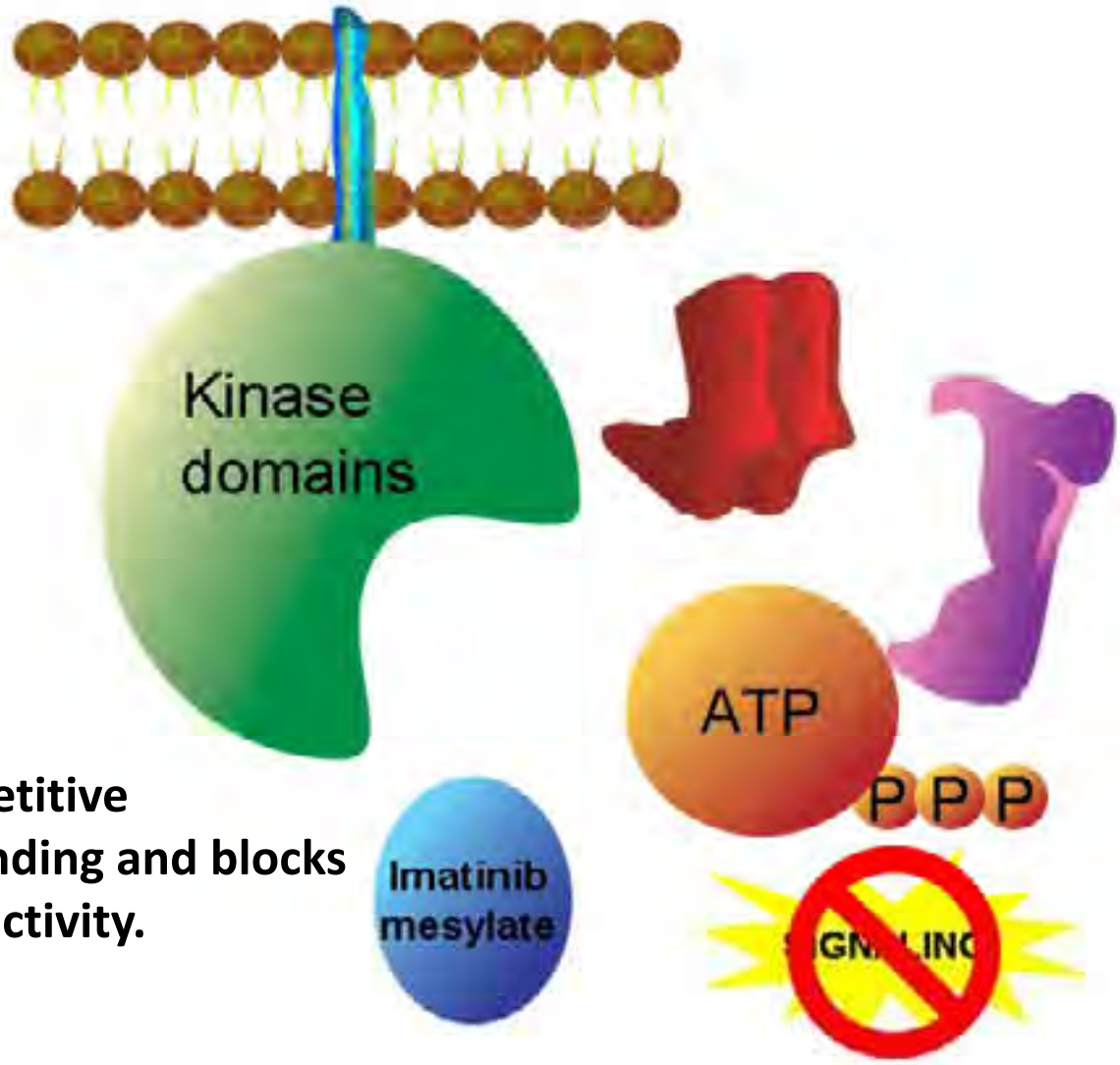
Activation of the substrate initiates a signaling cascade, culminating in cell **proliferation and survival**

ADP, adenosine diphosphate; ATP, adenosine triphosphate.

Savage et al. N Engl J Med. 2002;346:683-693.
Scheijen et al. Oncogene. 2002;21:3314-3333.

Inhibition of KIT Signaling by Imatinib

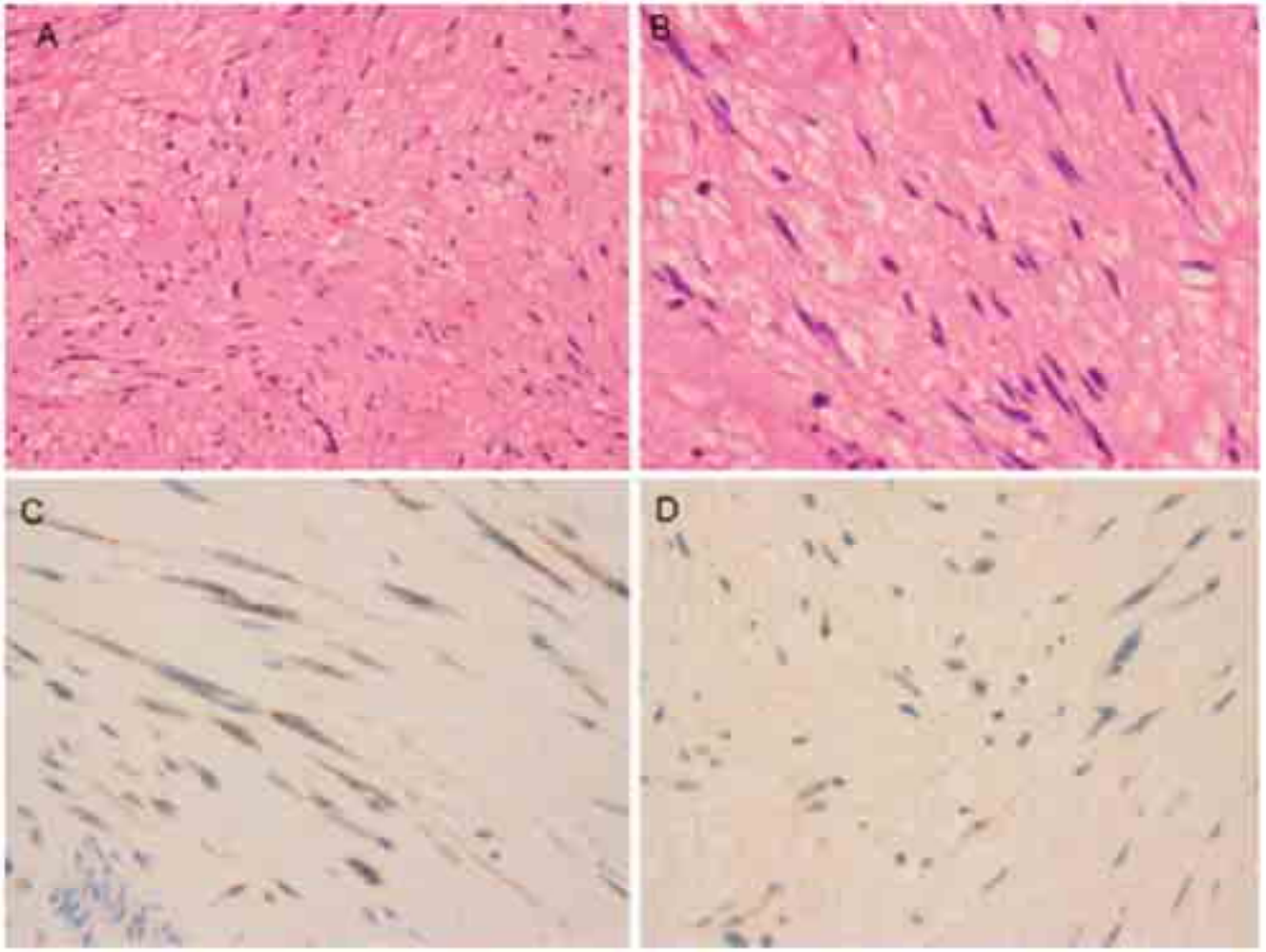
- The ATP binding pocket of the KIT kinase domain is occupied by imatinib
- Substrate phosphorylation is prevented and signaling is inhibited
- With signaling inhibited, proliferation and survival are interrupted



Imatinib is a competitive inhibitor of ATP binding and blocks kinase enzymatic activity.

Savage et al. N Engl J Med. 2002;346:683-693.
Scheijen et al. Oncogene. 2002;21:3314-3333.

Imatinib responsive GIST



Microscopic appearance of the liver metastasis from Figure 3, showing a low-cellularity spindle-cell GIST with increased fibrous stroma and no mitoses (A, B). The KIT expression was weak and focal (C), while the Ki67 proliferation index confirmed the lack of mitotic activity (D).

MILESTONES

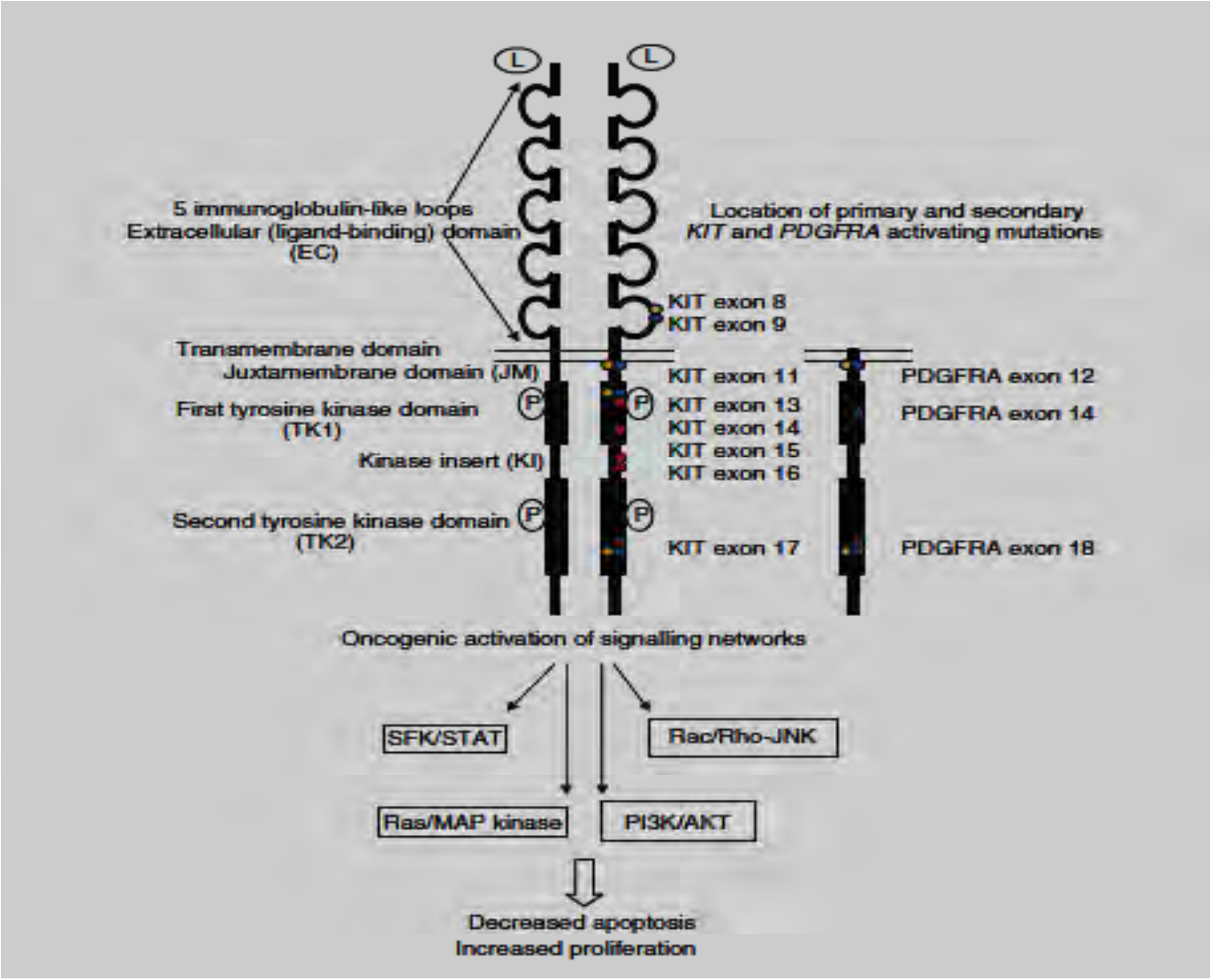
75-85% GISTs harbor activating mutations in KIT gene (Exon 11=65%; Exon 9=15%; Exon 13 and 17= 1,5%)

In ~10% of GISTs, the *KIT* gene is normal but there is a mutation in the platelet-derived growth factor receptor alpha gene,*PDGFRA*, a type III receptor tyrosine kinase, like KIT (Exon 18=4% mut; Exon 12= 0,8 mut; Exon 14= 0,3 mut).

Mutation in one of two receptor tyrosine kinase (RTK) genes (KIT and PDGFRA) is a key event in the pathogenesis of most GISTs.

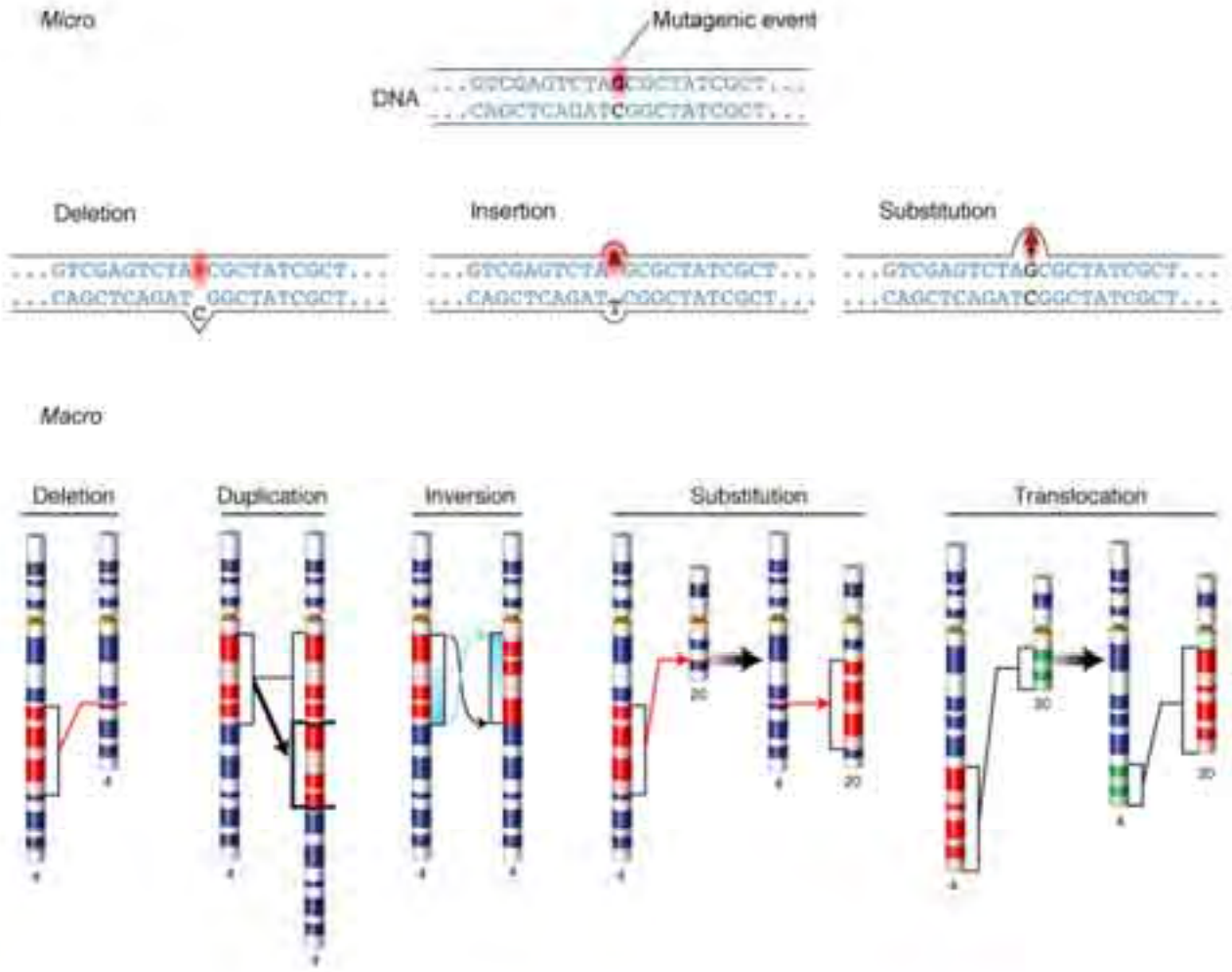
Subsequently, demonstration of a KIT or PDGFRA mutation is being used increasingly to supplement morphological and immunohistochemical assessment for diagnosing GIST, and has indeed been advocated as the gold standard for making such a diagnosis.

About 10% of GISTs are Wild Type (no KIT or PDGFRA mut.)

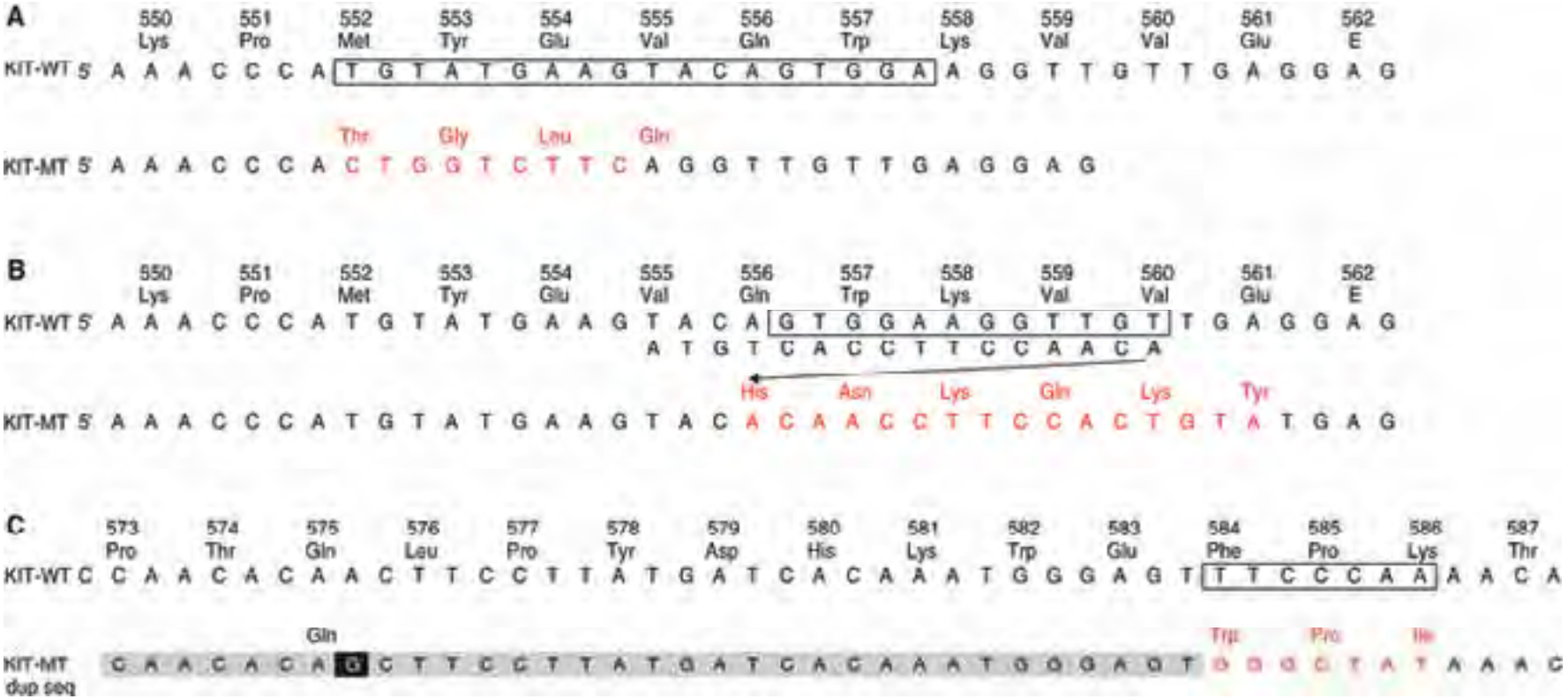


Based on the **location**, these mutations could be divided in:
1) mutations of the receptor regulatory domain (EC and JM)
2) mutations of the enzymatic domain (TK1 and TK2)

Mutation

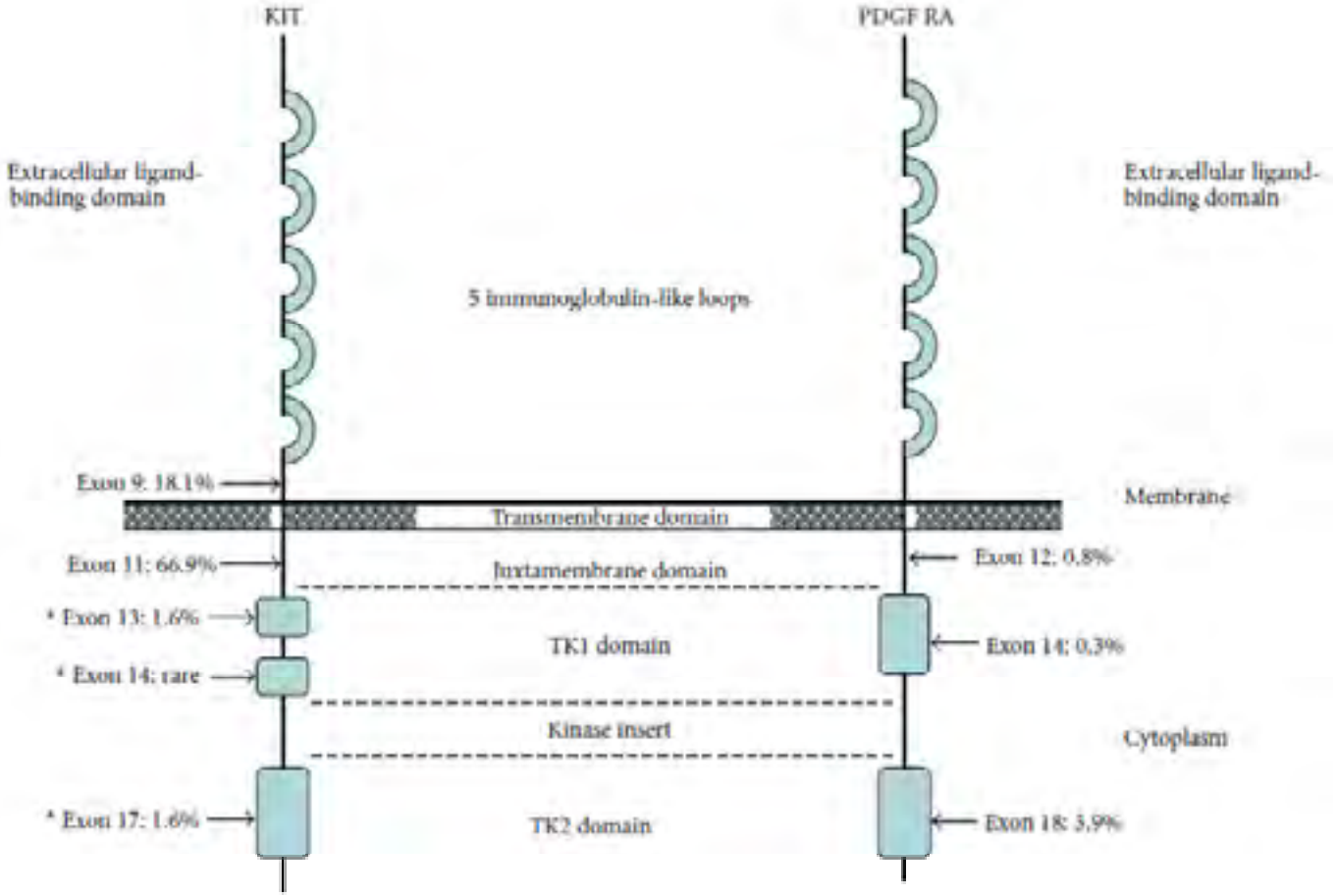

Micro
Mutagenic event
DNA
...GTCGAGTCTAGCGCTATCGCT...
...CAGCTCAGATCGGCTATCGCT...
Deletion
...GTCGAGTCTAGCGCTATCGCT...
...CAGCTCAGATCGGCTATCGCT...
C
Insertion
...GTCGAGTCTAGCGCTATCGCT...
...CAGCTCAGATCGGCTATCGCT...
T
Substitution
...GTCGAGTCTAGCGCTATCGCT...
...CAGCTCAGATCGGCTATCGCT...
Macro
Deletion
Duplication
Inversion
Substitution
Translocation

Clinical significance of oncogenic and mutations in gastrointestinal stromal tumours



Examples of complex KIT exon 11 mutations: **deletion–insertion** (A), **deletion–inversion** (B) and **duplication with deletion–insertion** (C). Deleted sequences are indicated by clear boxes on KIT-WT (wild-type). Inserted sequences are red in KIT-MT (mutant). Duplication is marked by a grey box. A silent mutation is indicated by a black box.

Histopathology (53), 3, 2008



Two Categories of KIT and PDGFRA Mutations in GIST

(i) mutations diagnosed in **primary tumours** before treatment with a TK inhibitor, linked to GIST pathogenesis: primary KIT and PDGFRA mutations

(ii) mutations **detected during treatment** causing resistance to imatinib-based TK inhibition: secondary KIT and PDGFRA mutations

Table 2. Summary of GIST clinicopathological features associated with *KIT* and *PDGFRA* mutations

Gene	Mutation at protein level	Clinicopathological features	Tumour type	Prognostic value
KIT-EC (exon 9)	Ala502_Tyr503dup	Strongly associated with intestinal GISTs (>90% of these mutations were identified in small intestinal tumours)	Predominantly spindle cell tumours	No prognostic value in intestinal GISTs
KIT-JM (exon 11)	Trp557_Lys558del	Occur in GISTs from different parts of GI tract	Spectrum of spindle cell and epithelioid tumours	May indicate more malignant behaviour, especially in gastric GISTs
	Deletions Deletion–insertions			
	Substitutions			May indicate less malignant behaviour in gastric GISTs
	Duplications	Associated with gastric GISTs	Predominantly spindle cell tumours	May indicate less malignant behaviour in gastric GISTs
KIT-TK1 (exon 13)	Lys642Glu	Occur in GISTs from different parts of GI tract		May indicate more malignant behaviour in gastric GISTs
KIT-TK2 (exon 17)	Asn822Lys	Two times more frequent in intestinal GISTs		No prognostic value
PDGFRA-JM (exon 12)	Deletions Substitutions	Strongly associated with gastric GISTs (>95% of such mutations identified in tumours from stomach)	Predominantly epithelioid or mixed epithelioid and spindle cell tumours	May indicate less malignant behaviour in gastric GISTs
PDGFRA-TK1 (exon 14)	Substitutions			
PDGFRA-TK2 (exon 18)	Deletions Substitutions			
KIT PDGFRA	Wild-type	Occur in GISTs from different parts of GI tract	Spectrum of spindle cell and epithelioid tumours	No prognostic value
		GISTs in NF1 (intestinal tumours)	Almost exclusively spindle cell tumours	No prognostic value
		GIST in Carney triad and paediatric GISTs (gastric tumours)	Predominantly epithelioid tumours	

Table 5. KIT and PDGFRA genotypes, *in vitro* sensitivity to imatinib and response to imatinib treatment based on previously published studies from US and European clinical trials

Gene	Exon	Primary *KIT* and *PDGFRA* mutations identified in GISTs from imatinib clinical trials (*n*)	Sensitivity to imatinib mesylate
KIT	9	Ala502_Tyr503dup	Sensitive to imatinib *in vitro*[29] Complete remission in 5%, partial response in 29%, stable disease in 47%, progressive disease in 17% as reported by EORTC phase III trial[84] A high-dose regimen increased progression-free survival[84]
	11	Deletion/deletion–insertion Substitution Duplication	Most common mutants sensitive to imatinib *in vitro*[29] Rare Val559Ile mutant resistant to imatinib *in vitro*[27] Complete remission in 8%, partial response in 61%, stable disease in 25%, progressive disease in 3% as reported by EORTC phase III trial[84]
	13	Lys642Glu (8) Glu635Lys (1)	Sensitive to imatinib *in vitro*[29] Partial response or stable disease reported in all nine cases[29,35,84]
	17	Asp820Tyr (1) Asn822Lys (2) Asn822His (2)	Asn822Lys and Asn822His sensitive to imatinib *in vitro*[29] Partial response reported in four mutants including Asn820Tyr, Asn822Lys, Asn822His[29,84] Primary resistance reported in Asn822Lys mutant[27]
PDGFRA	12	Asp561Val (4) Deletion/deletion–insertion Duplication, insertion	Asp561Val and some other exon 12 mutants tested sensitive to imatinib *in vitro*[30,111] Objective response reported in the majority of a few cases treated with imatinib[29,84]
	14	Asn659Lys	This mutant tested sensitive to imatinib *in vitro*[30] No clinical experience
	18	Asp842_His845del (2) Asp842_Met844del (1) Ile843del (1) Ile843_His845del (1) Asp842Val (7) Asp846Val (1)	Some of these and similar mutants tested sensitive to imatinib *in vitro*[30,111] Objective response reported in the majority of a few cases treated with imatinib[29,84] Asp842Val resistant to imatinib *in vitro*[29,30,111] Resistance reported in seven cases including Asp846Val[29,35,84], stable disease in one case after 5 months of imatinib treatment[35]
KIT PDGFRA	9, 11, 13, 17 12, 14, 18	Wild-type Wild-type	Partial response in 23%, stable disease in 50%, and progressive disease in 19% as reported by EORTC phase III trial[84]

GIST, Gastrointestinal stromal tumour; PDGFRA, platelet-derived growth factor-alpha

MAJOR POINTS OF INTEREST

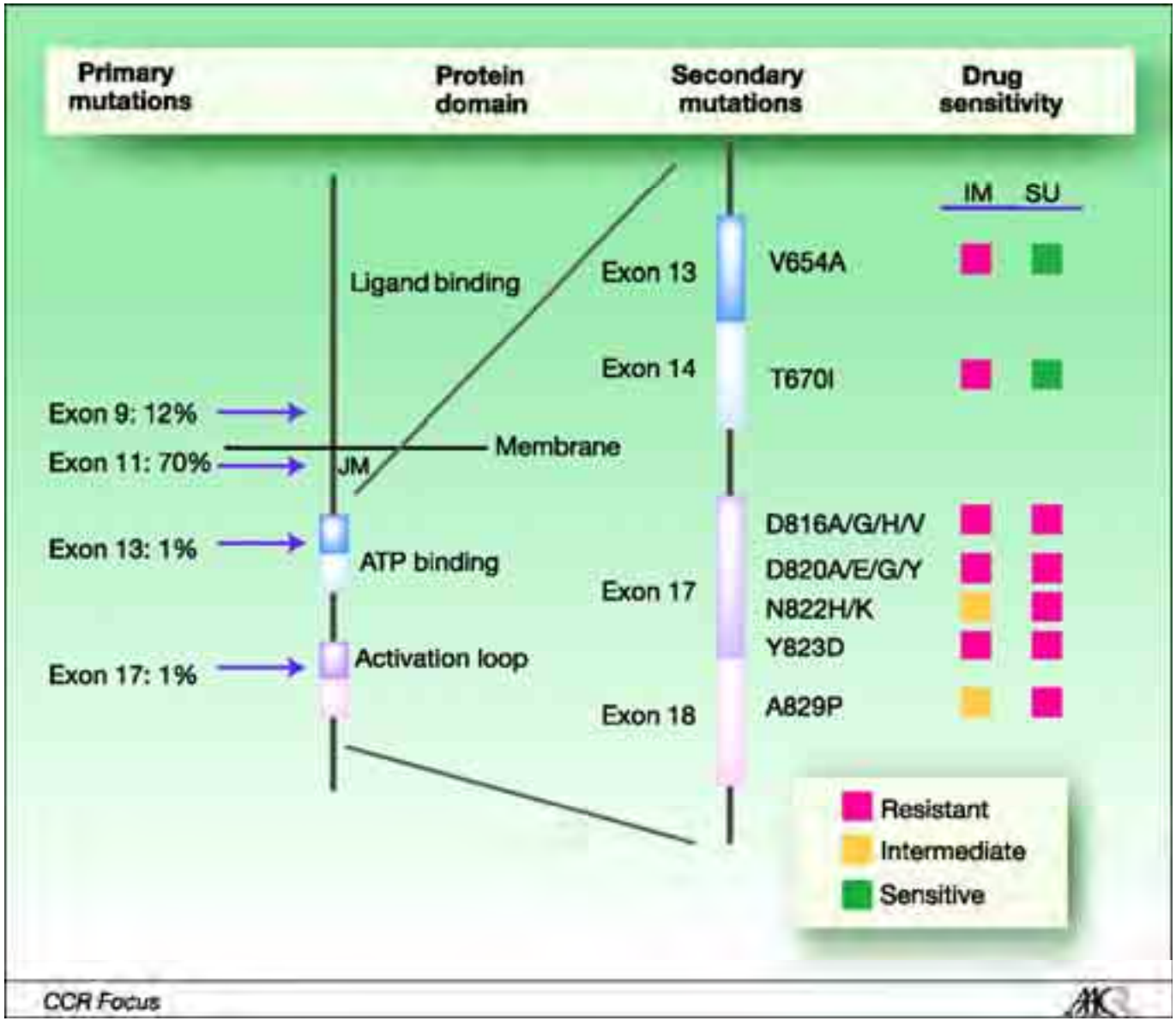
KIT **exon 11** mutations predict for a better in vivo primary response to imatinib than KIT **exon 9** mutations, a wild-type genotype and certain PDGFRA exon 18 mutations (particularly the D842V mutation)

While KIT exon 9 mutations produce relative resistance to imatinib therapy, this resistance might be overcome by dose escalation (from 400 to 800 mg daily)

The genotype profile associated with primary resistance to sunitinib is almost the opposite of that for imatinib: KIT exon 9 mutations and a wild-type genotype predict for a better response to sunitinib than KIT exon 11 mutations

However, there are some mutations, in particular the **PDGFRA D842V** mutation which predict for primary resistance to both imatinib and sunitinib, at least in the in vitro setting

Location and biochemical properties of secondary KIT kinase mutations in TKI-resistant GIST. The location of primary KIT kinase mutations is shown on the left-most stick figure.



Gramza A W et al. Clin Cancer Res 2009;15:7510-7518

PRIMARY and DELAYED IMATINIB RESISTANCE

Most GIST patients (65% to 70%) respond to imatinib, and another 15% to 20% obtain disease stabilization.
Approximately 15% of all GISTs are primarily resistant to imatinib and thus the tumor continues to grow.
Causes of primary resistance: 1) KIT/PDGFRa wild type; 2) Exon 9 mutation; 3) PDGFRa D842V substitution

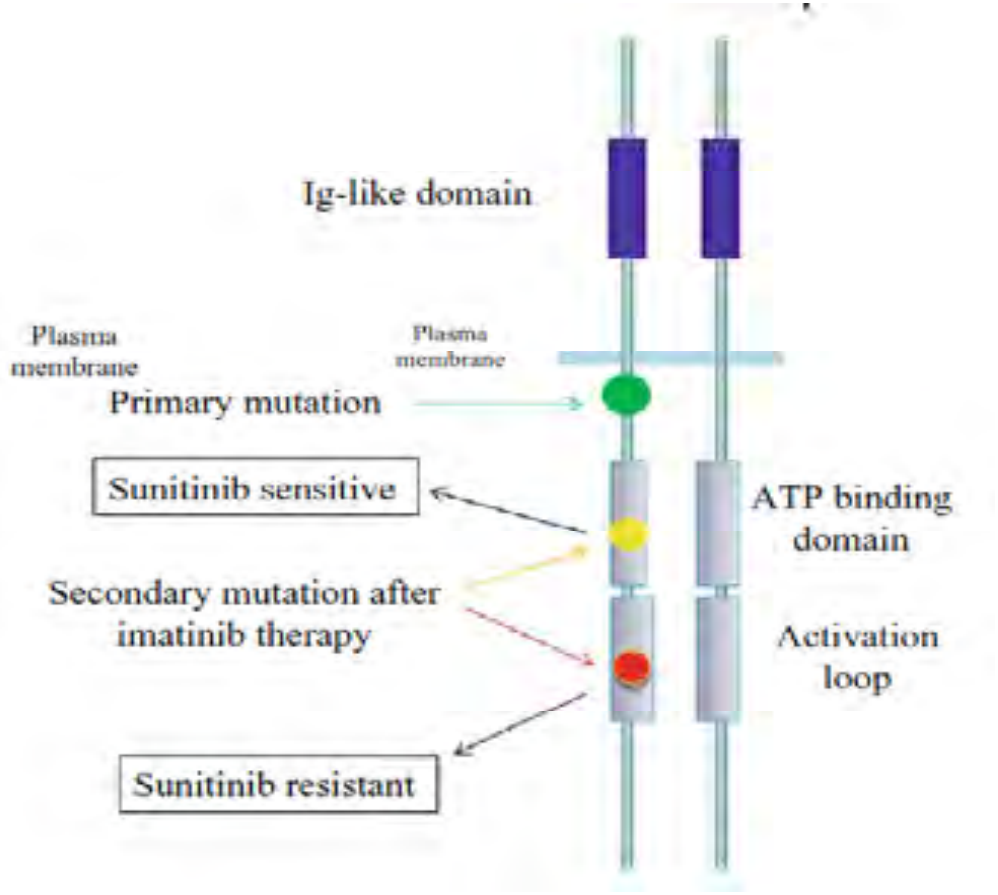
An additional 50% of patients will go on to develop imatinib resistance within 2 years, after enjoying a partial response or at least disease stabilization during initial follow-up.
These patients are classified as having **delayed resistance**.

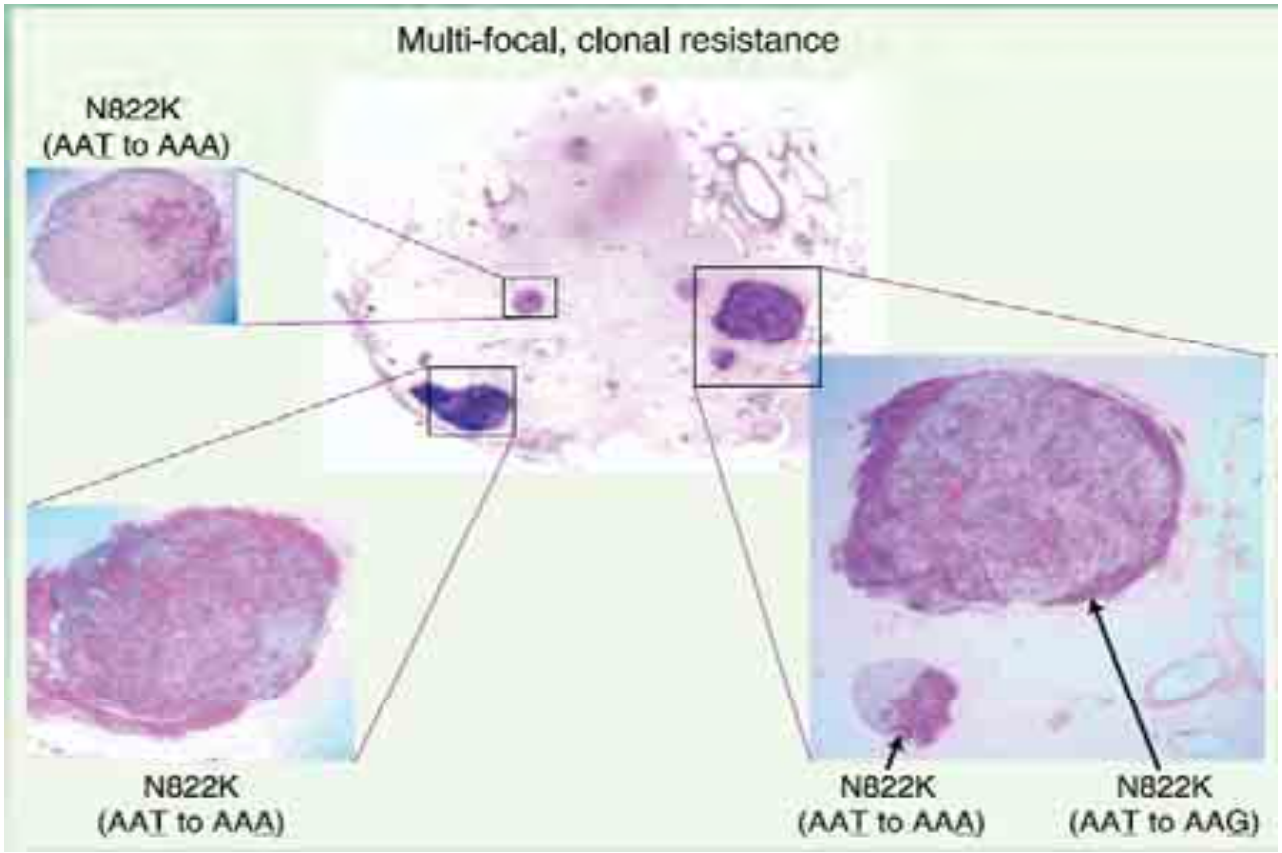
Delayed (secondary) resistance is mainly due to: i) secondary mutations of the *KIT* or *PDGFRA* gene (70%–80%); ii) overexpression of KIT and/or an increase in the copy number of mutated *KIT* (10%); iii) gain of new but unknown proliferation mechanisms with a concomitant loss of KIT control (10%).
Secondary mutations and overexpression of KIT are generally termed "**target resistance**" and the last resistant mechanism with other newly activated systems is generally termed "**biological resistance**", where KIT expression has disappeared and tumors show the morphological appearance of rhabdomyoblastic differentiation including the expression of desmin

The **incidence** of secondary mutations was greater in GISTs with initially imatinib sensitive mutations, i.e., GISTS with *KIT* exon 11 and *PDGFRA* exon 12 mutations, than in GISTs with the less-sensitive mutation, *KIT* exon 9

Secondary mutation in the kinase domains is accompanied by concomitant **re-activation** of the corresponding tyrosine kinase even in the presence of imatinib.

Secondary mutations also have **hot spots**, including *KIT* exon 13 (codon 654); exon 14 (codon 670); exon 17 of codons 809, 816, 820, 823, and 829; and *PDGFRA* exon 14 and exon 18

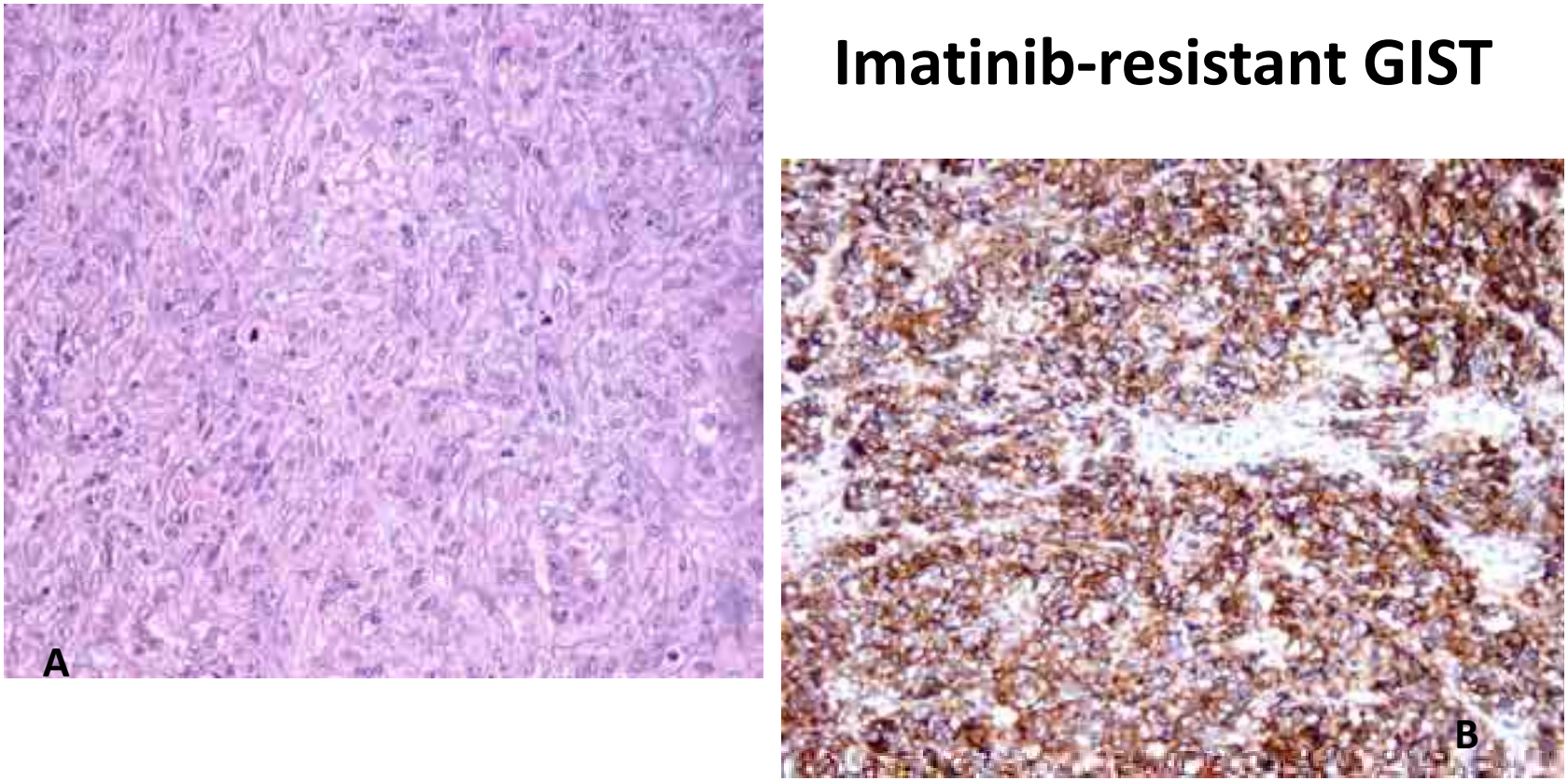




Histologic and molecular correlates of **multifocal clonal resistance**. A GIST lesion was surgically removed from a patient who responded to imatinib. The lesion was largely acellular, but harbored **four microscopic foci** of viable tumor, each of which had a secondary mutation in KIT exon 17 (N822K). Interestingly, there was **mutational heterogeneity** in the N822K mutations evident at the nucleotide level (AAT → AAA in three lesions, AAT → AAG in top right lesion). It is presumed that each of these foci represents an independent clone of resistant cells.

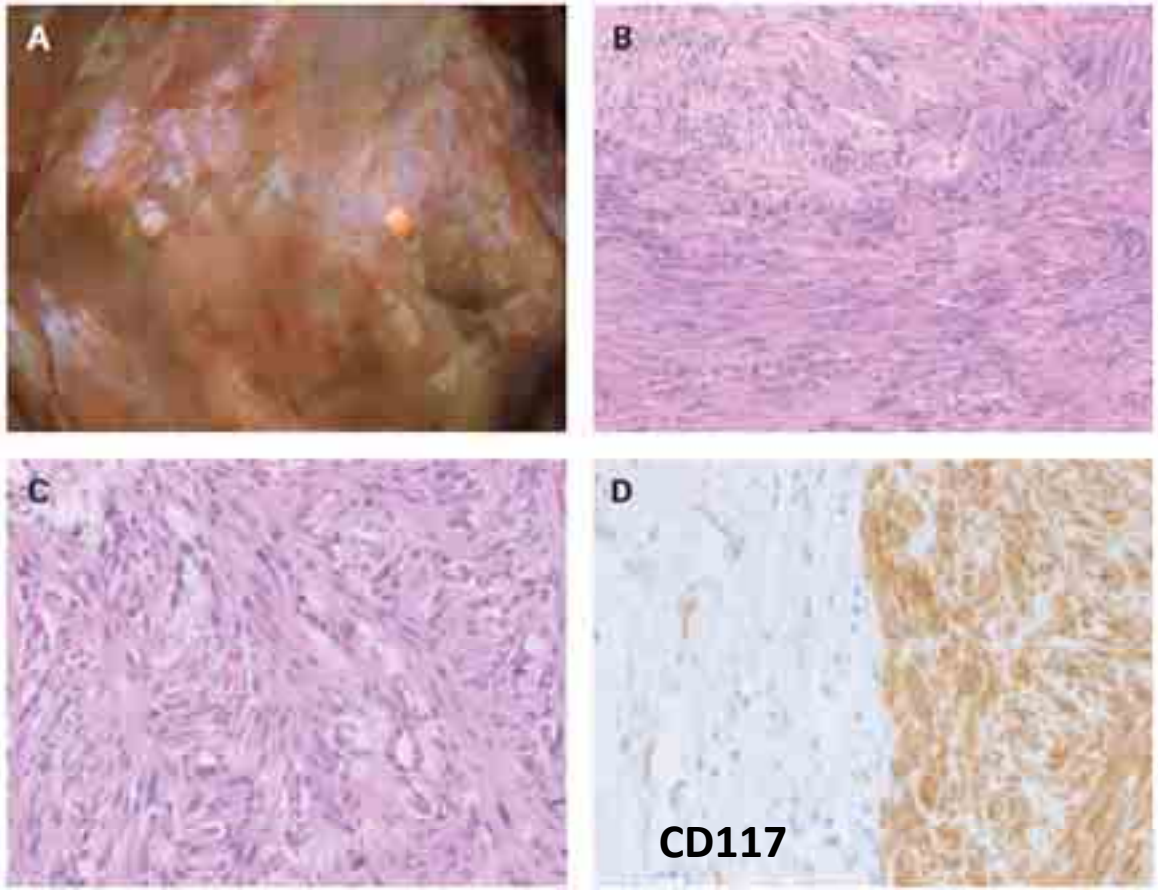
Gramza 2009

Imatinib-resistant GIST



Microscopic appearance of an imatinib-resistant GIST, showing high cellularity and mitotic activity (A); KIT expression was diffuse and strong (B), while Ki67 showed a high proliferation index.

V600E BRAF mutations are alternative early molecular events in a subset of KIT/PDGFRA wild type gastrointestinal stromal tumours

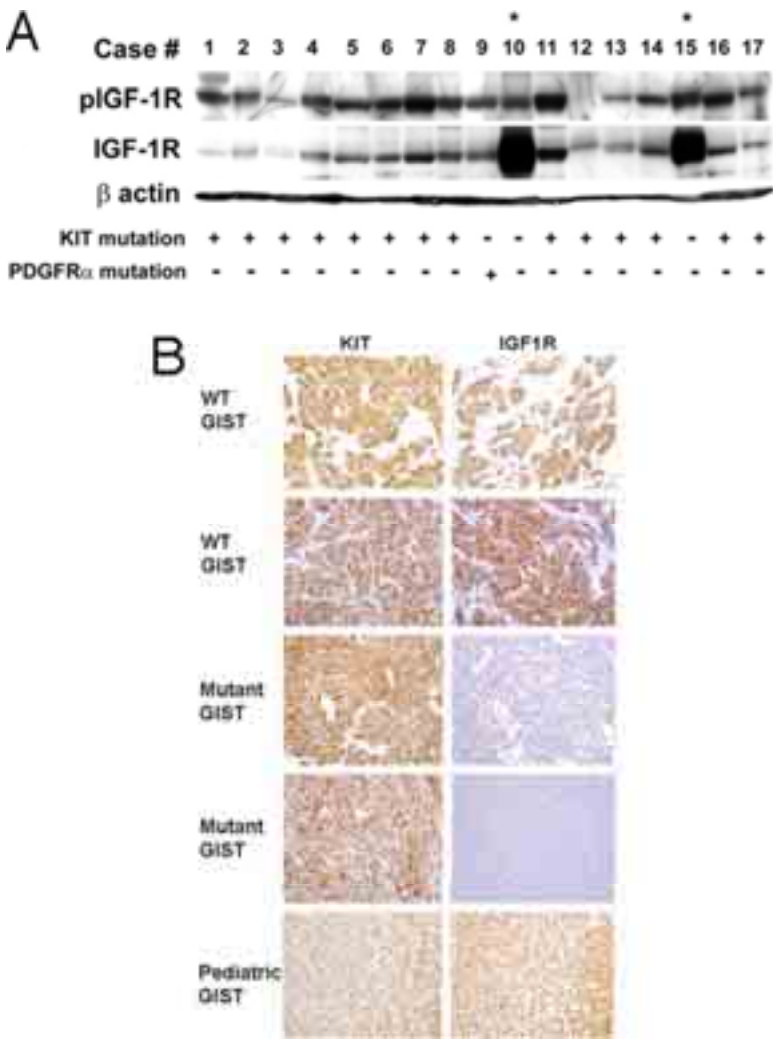


V600E BRAF mutation is present in 4% of KIT/DGFRA wild-type GISTs

None of KIT/PDGFRA-mutated GISTs revealed a BRAF mutation indicating that these mutations are **mutually exclusive**.

Terracciano et al *J Clin Pathol 2009;62*

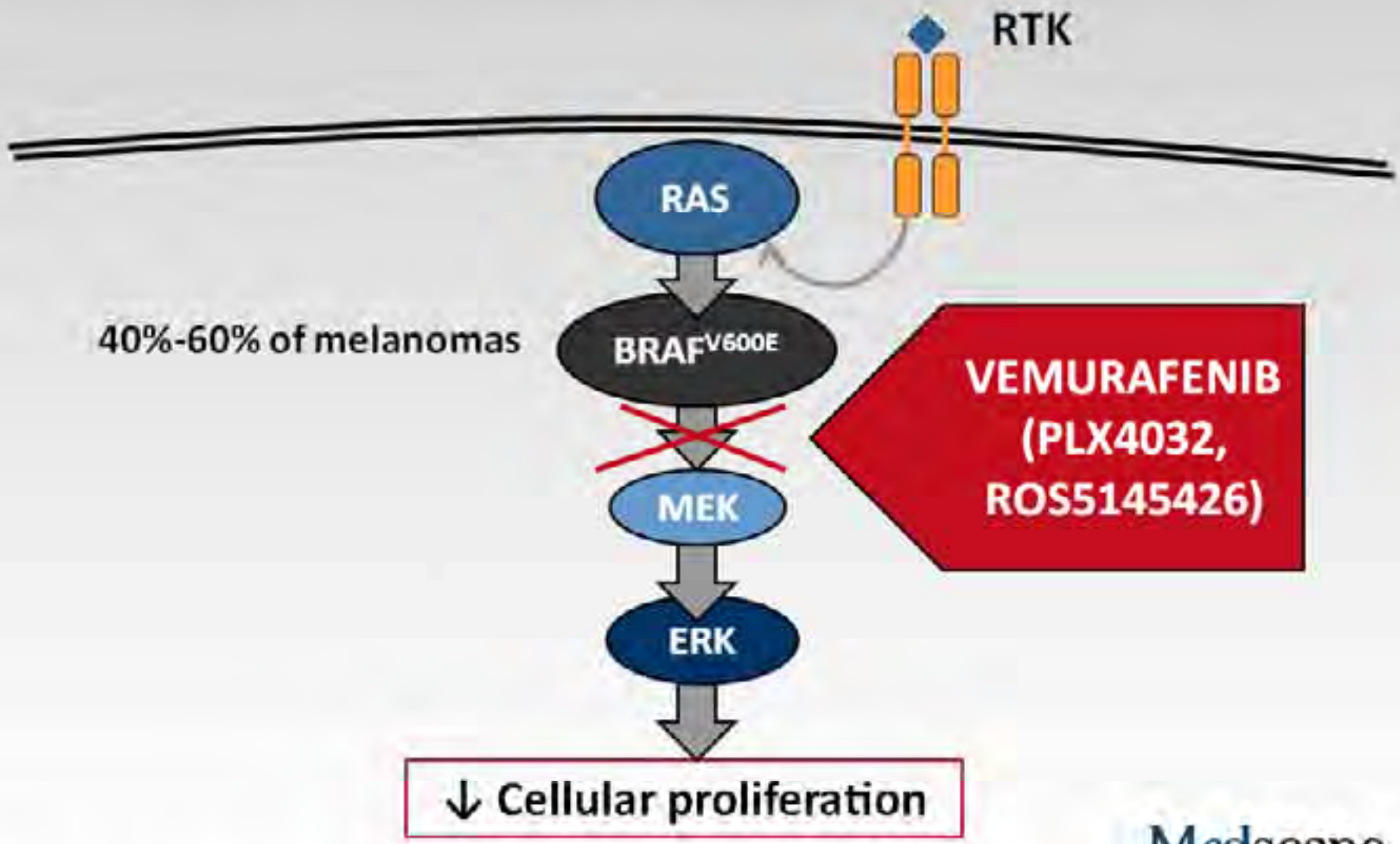
IGF1R expression in GIST biopsies.



Tarn C et al. PNAS 2008;105:8387-8392

TREATMENTS UNDER INVESTIGATION

Vemurafenib Inhibits BRAF^{V600E} Kinase



Chapman PB, et al. ASCO; 2011. Abstract LBA4.

Medscape EDUCATION

Regorafenib in GIST Following Failure of Imatinib and Sunitinib: Overall PFS

Objective response	N = 33 n (%)
PR	3 (9)
SD	
≥ 16 weeks	21 (64)
< 16 weeks	6 (18)
PD	2 (6)
Not evaluable	1 (3)
Median PFS	10.0 months (95% CI: 7.3, ...)
OS	not yet reached

Clinical benefit
n = 24 (73%)
95% CI: 55%-87%

PD = progressive disease

George S, et al. ASCO; 2011. Abstract 10007

Medscape EDUCATION

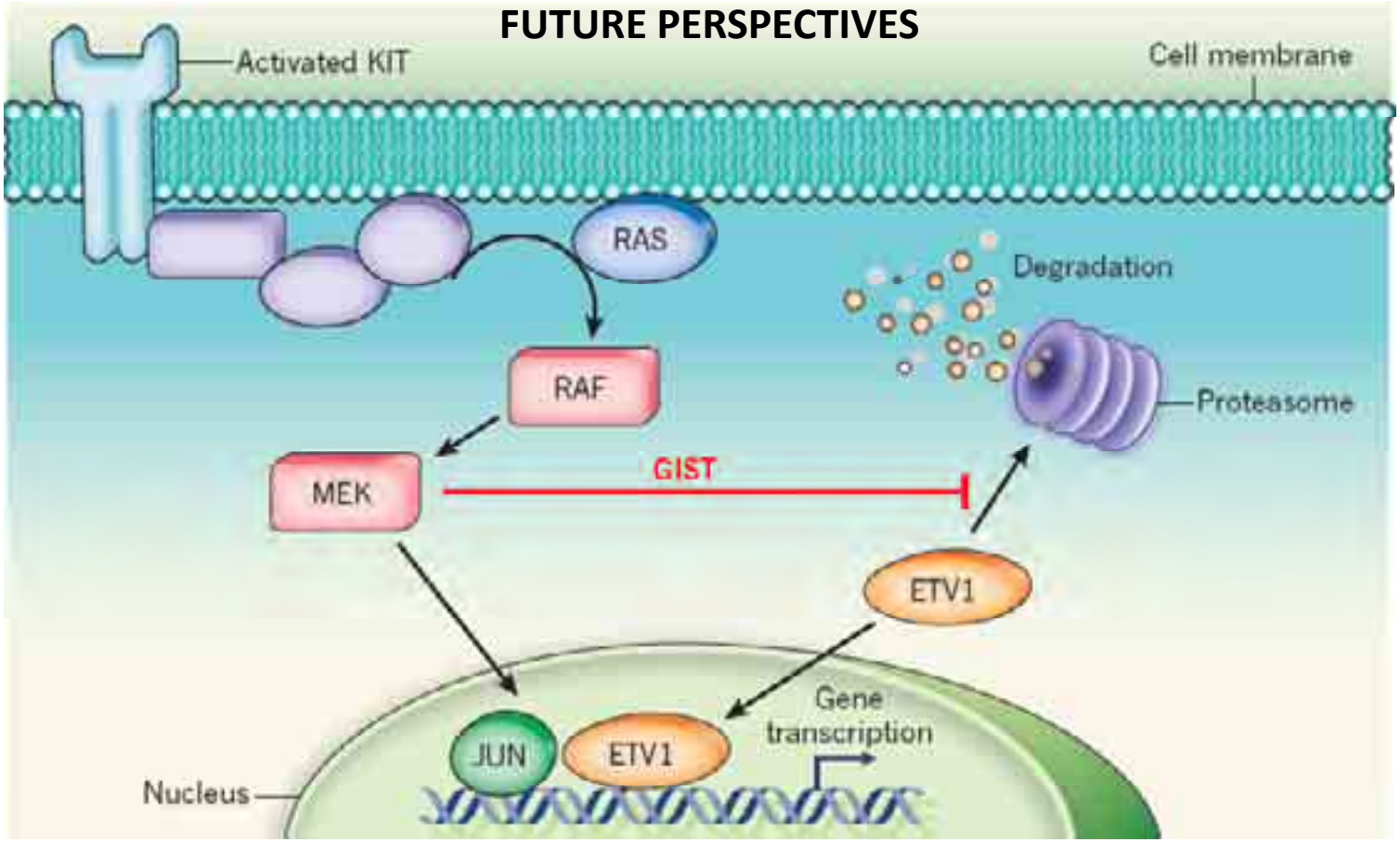
Crenolanib (CP 868-956)

- Crenolanib blocks PDGFRA D842V at clinically achievable concentrations, even in the presence of the T6741 mutation[a]
- Crenolanib may be effective for GIST patients with primary or secondary PDGFRA D842V mutations who are resistant to imatinib, sunitinib, and other TKIs[a]
- Phase 2 trial of crenolanib in GIST patients with PDGFRA D842V mutation is currently enrolling patients[b]

a. Heinrich M, et al. ASCO; 2011. Abstract 10012.
b. Clinicaltrials.gov: NCT01243346.

Medscape EDUCATION

FUTURE PERSPECTIVES



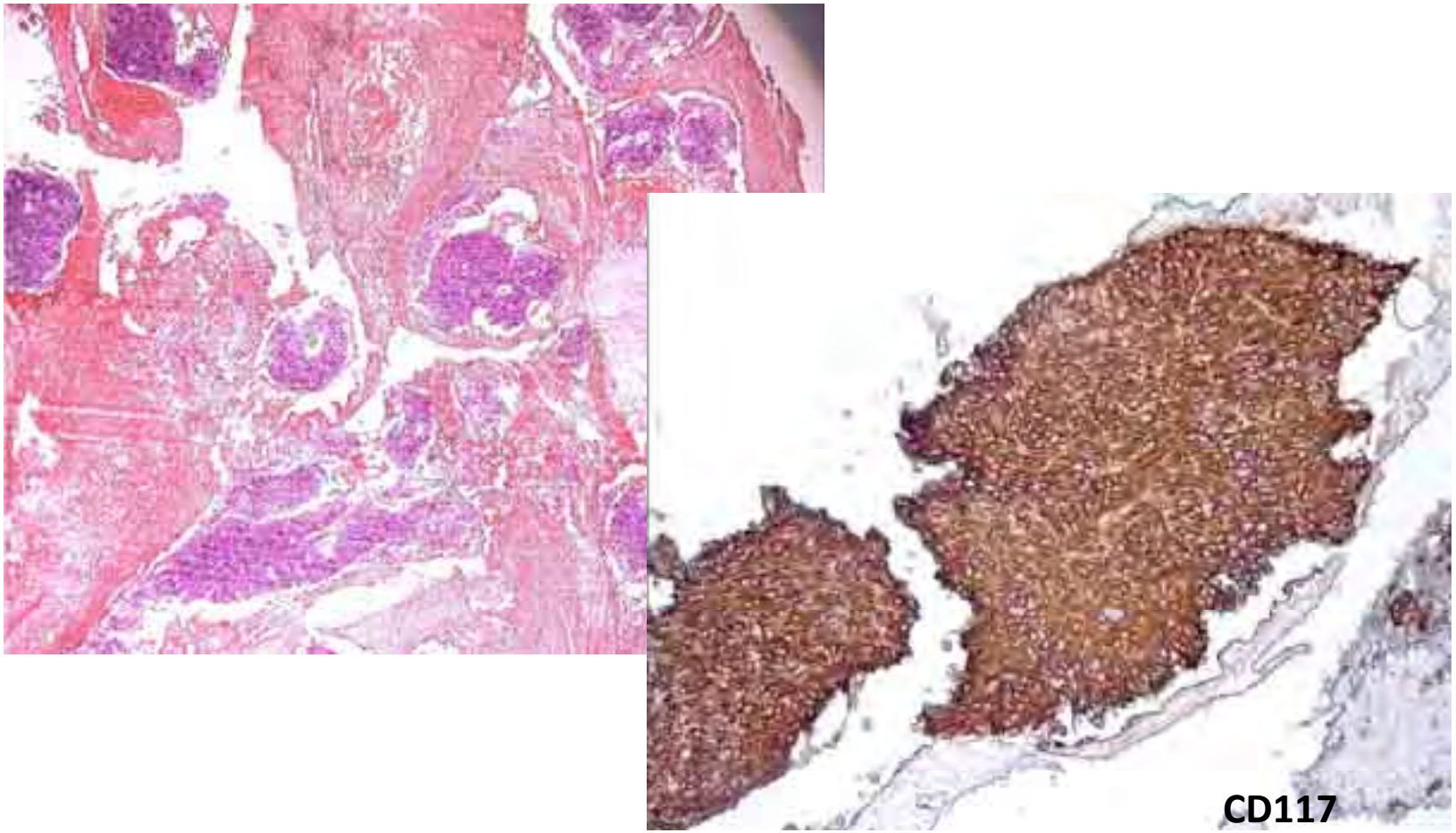
The **transcription factor ETV1** acts downstream of the RAS/RAF/MEK pathway and can directly regulate **gene expression**. Families harbouring germline activating *KIT* mutations and mice with knock-in *Kit* mutations almost exclusively develop ICC hyperplasia and GIST suggesting that the cellular context is important for *KIT* to mediate oncogenesis. The *ETS* family member *ETV1* is highly expressed in the subtypes of ICCs sensitive to oncogenic *KIT* mediated transformation[8], and is required for their development.

Chi et al. Nature 2010

WHAT AM I DOING HERE?

B.Chatwin 1988

…..THE DIAGNOSIS



Histology and immunohistochemistry remain a crucial tool, particularly for distinguishing GIST from its mimics

"...The pathologist has the unique opportunity of bridging the gap between the manifestations of disease and its causes, and he should take advantage of this circumstance making fundamental contributions to knowledge..."

L. V. Ackerman