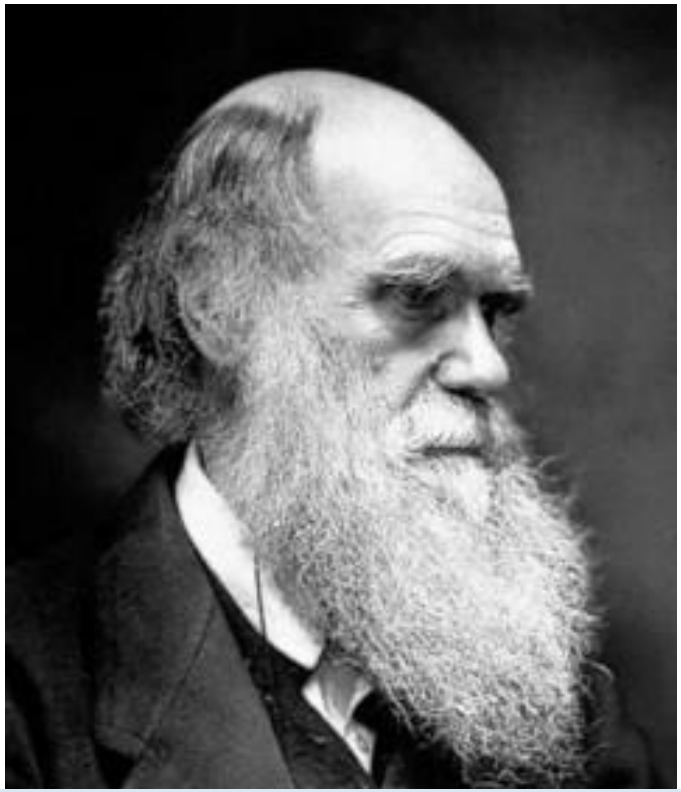
The Relevance of Genetics and Genotype to the Clinical Management of GIST

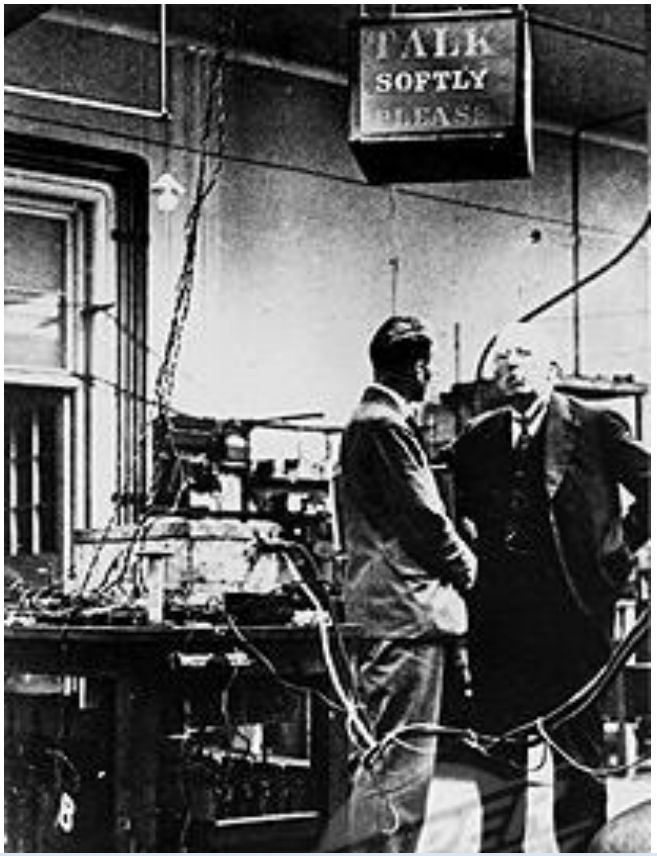
Mohamad Farid
National Cancer Centre Singapore
12 Sept 2015

The knotty nature of classification



This classification is evidently not arbitrary like the grouping of stars into constellations.

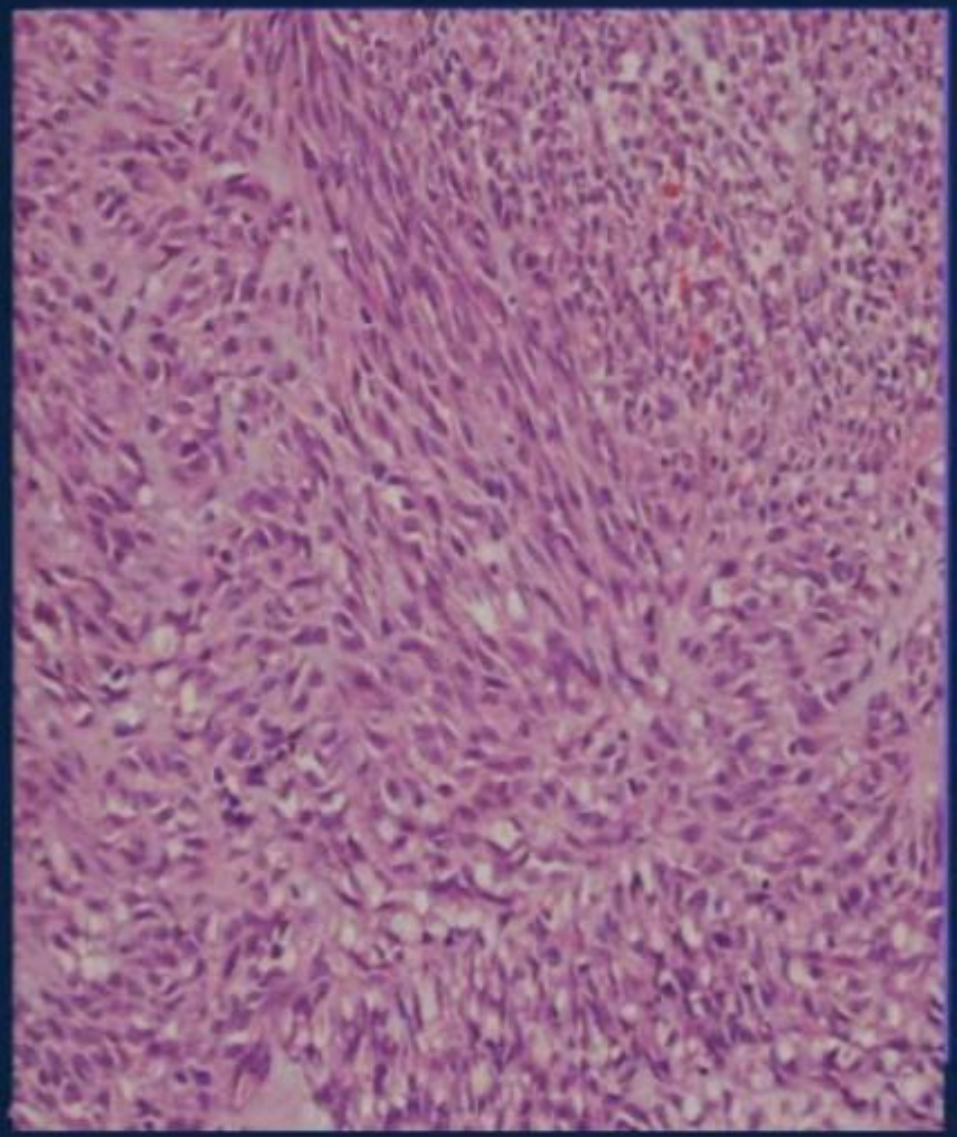
Charles Darwin (1809 – 1882)

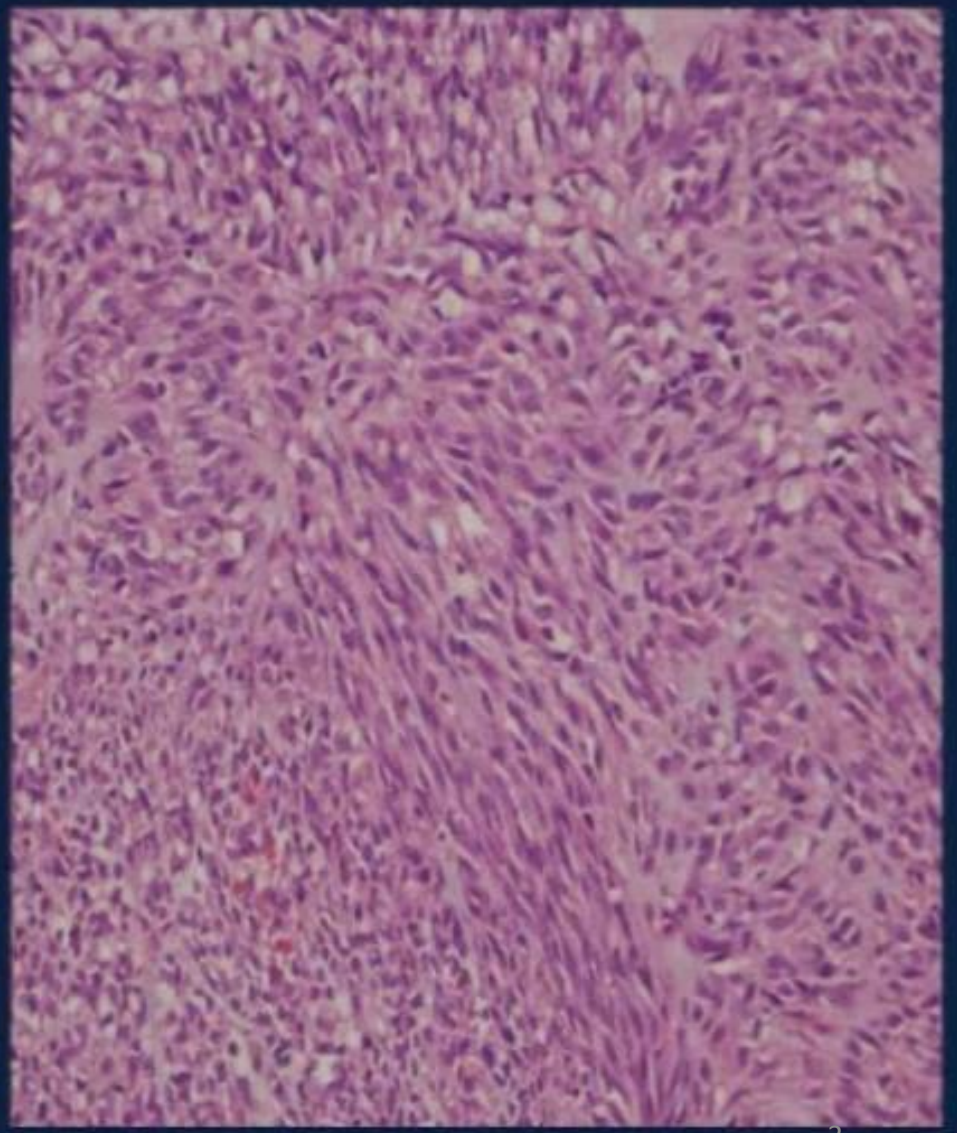


All of science is either physics or stamp collecting.

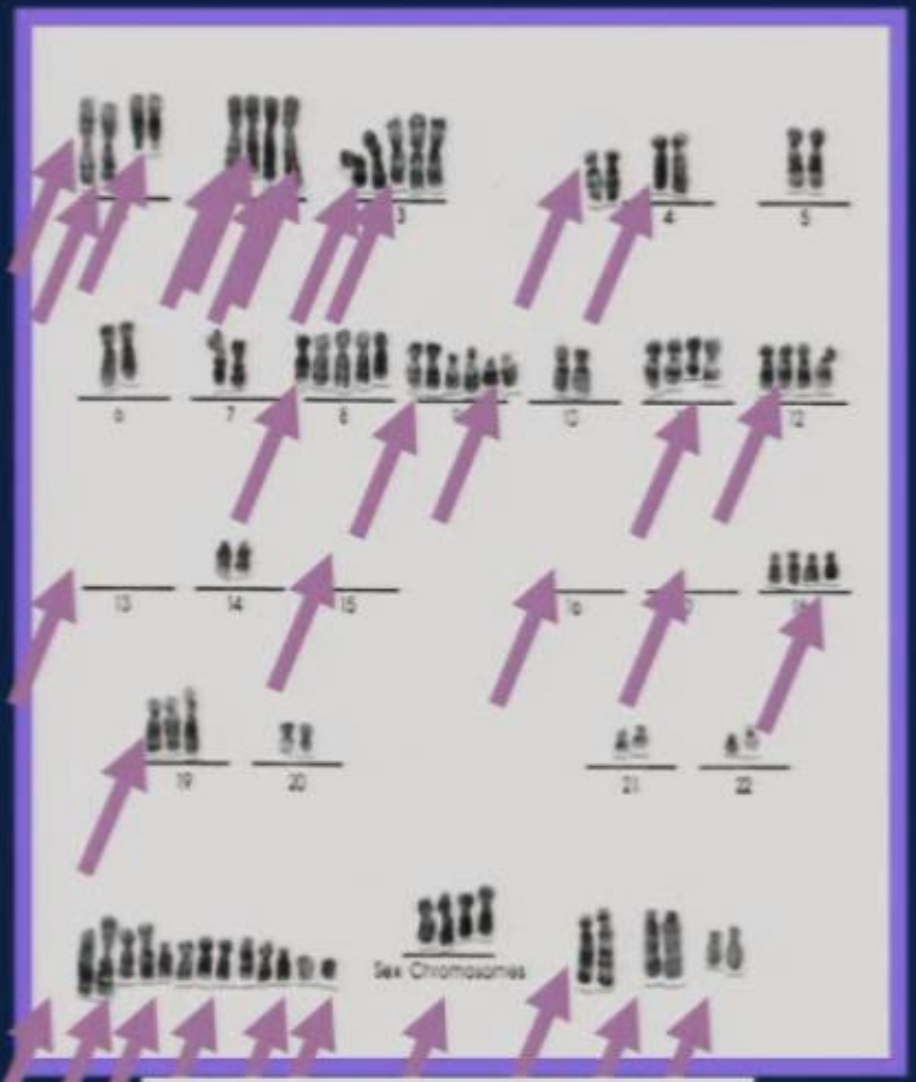
Ernest Rutherford (1871 – 1937)

Same Disease? Or Completely Different?

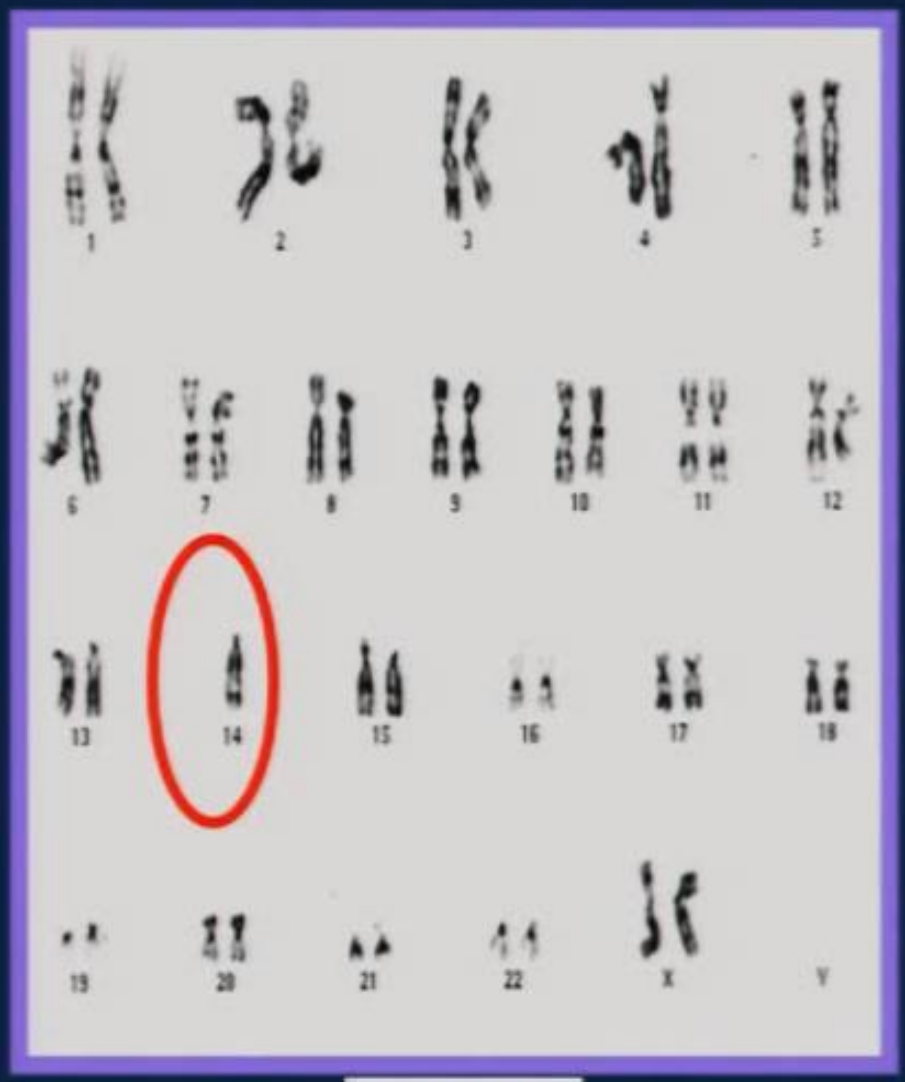




Same Disease? Or Completely Different?



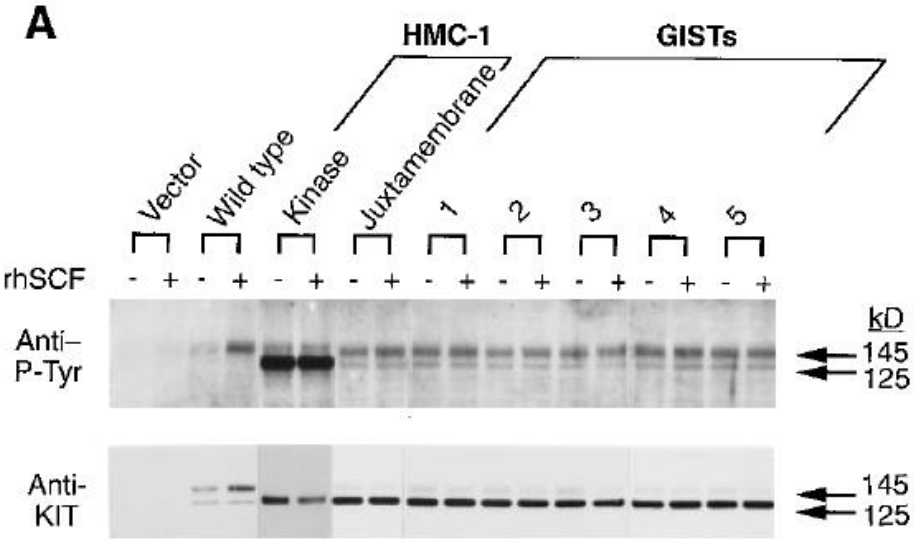
Leiomyosarcoma



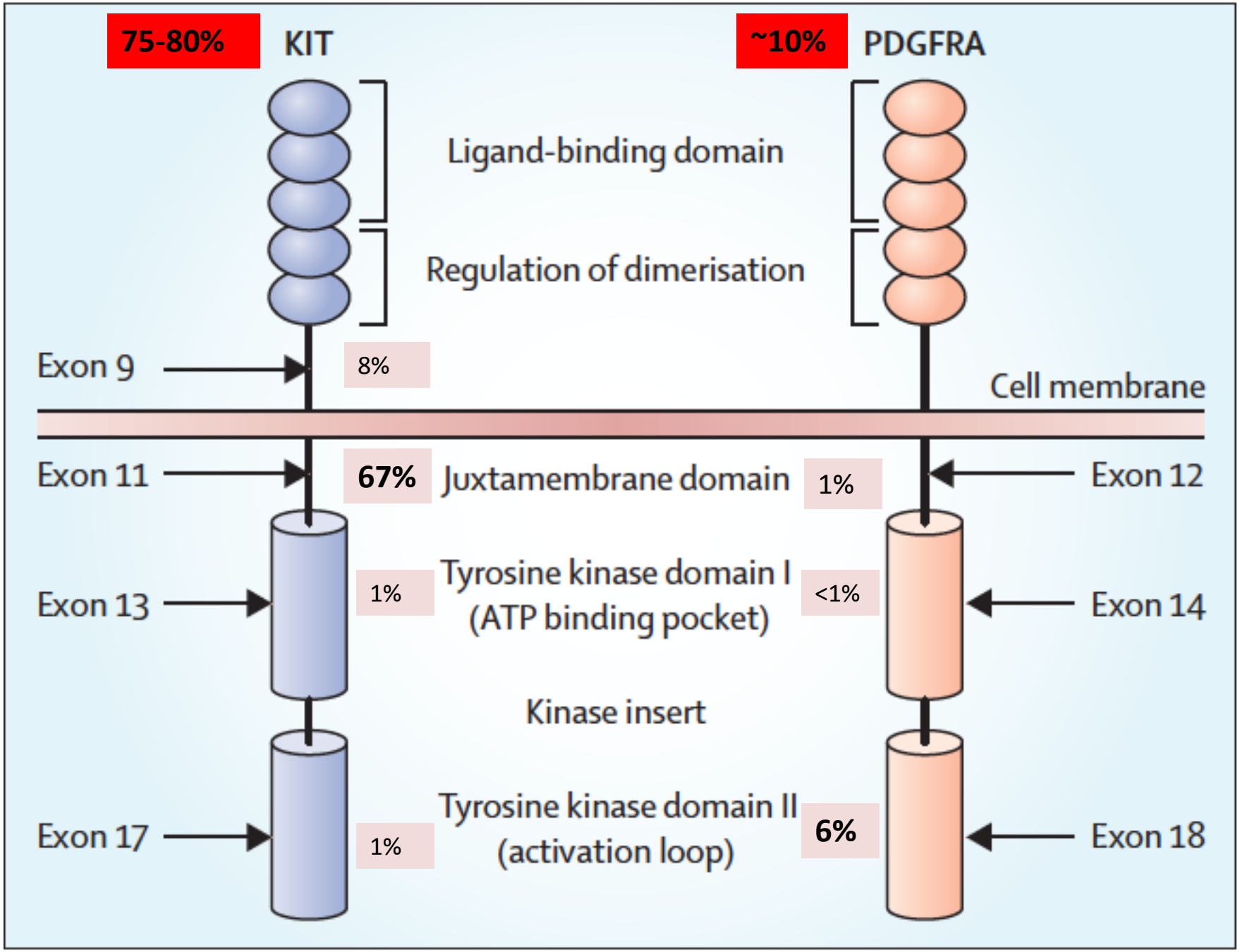
GIST

Gain-of-Function Mutations of c-*kit* in Human Gastrointestinal Stromal Tumors

Seiichi Hirota,* Koji Isozaki,* Yasuhiro Moriyama, Koji Hashimoto, Toshirou Nishida, Shingo Ishiguro, Kiyoshi Kawano, Masato Hanada, Akihiko Kurata, Masashi Takeda, Ghulam Muhammad Tunio, Yuji Matsuzawa, Yuzuru Kanakura, Yasuhisa Shinomura, Yukihiko Kitamura†



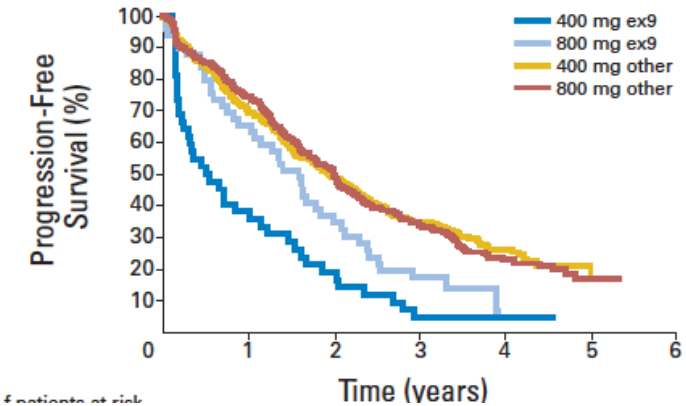
Hirota et al Science 1998



Joenssu et al Lancet 2013

Genotype –Phenotype Correlates

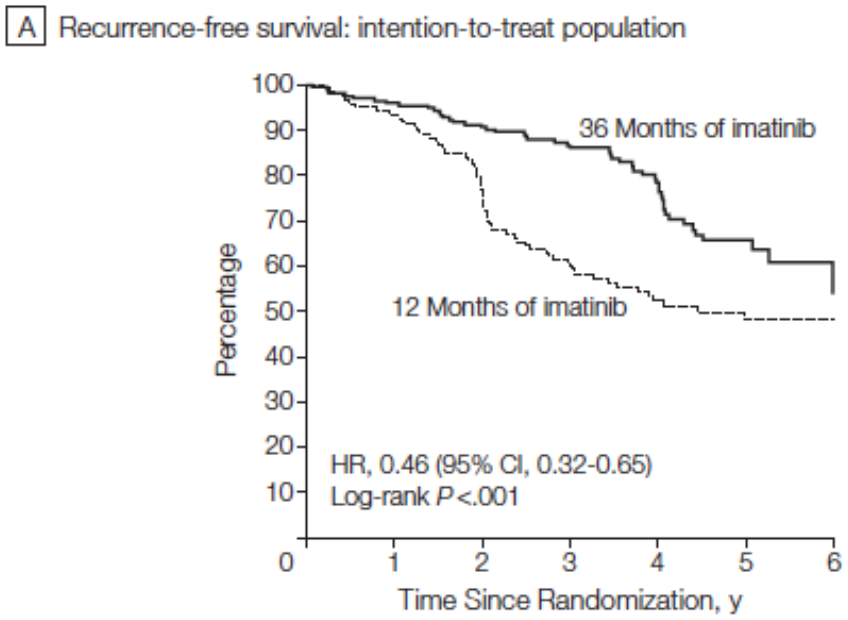
Genetic Type	Relative Frequency (%)	Anatomic Distribution	Notable Features
KIT mutation	77	—	—
Exon 8	Rare	Small bowel	
Exon 9	8	Small bowel, colon	Better responses higher-dose imatinib
Exon 11	67	All sites	Respond well to imatinib
Exon 13	1	All sites	Imatinib responsive
Exon 17	1	All sites	Many are imatinib sensitive
PDGFRA mutation	10	—	—
Exon 12	1	All sites	Sensitive to imatinib
Exon 14	<1	Stomach	Sensitive to imatinib
Exon 18 D842V	5	Stomach, mesentery, omentum	Imatinib resistant
Exon 18 other	1	All sites	Some but not all are imatinib sensitive

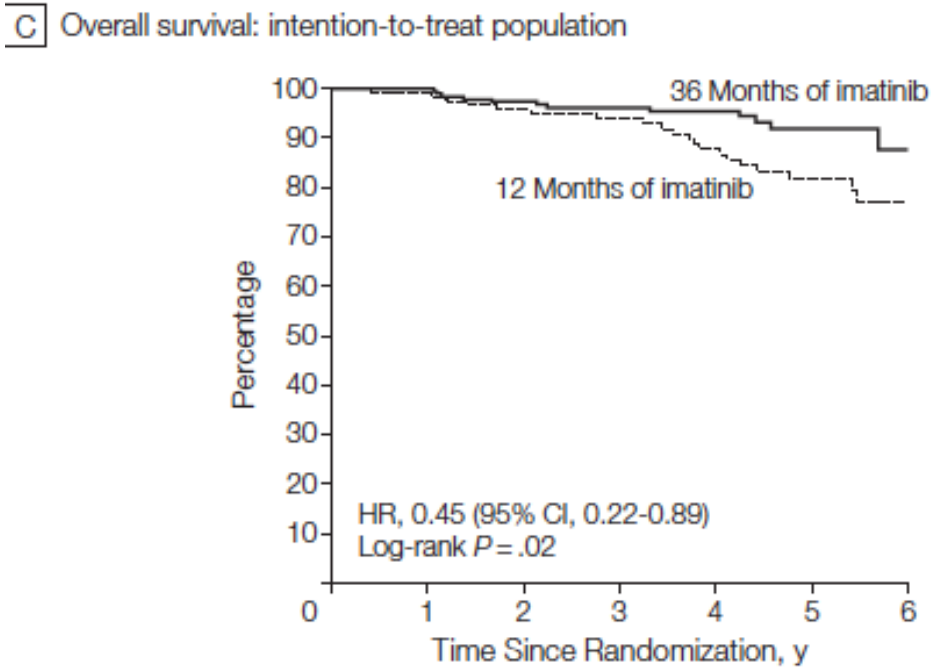


METAGIST JCO 2010

Barnett et al HOCNA 2013

Adjuvant Imatinib Improves Survival in High Risk GIST





RFS **+20%**

5-year RFS, 65.6% vs 47.9%,
HR, 0.46; 95% CI; 0.32 - 0.65
P<.001)

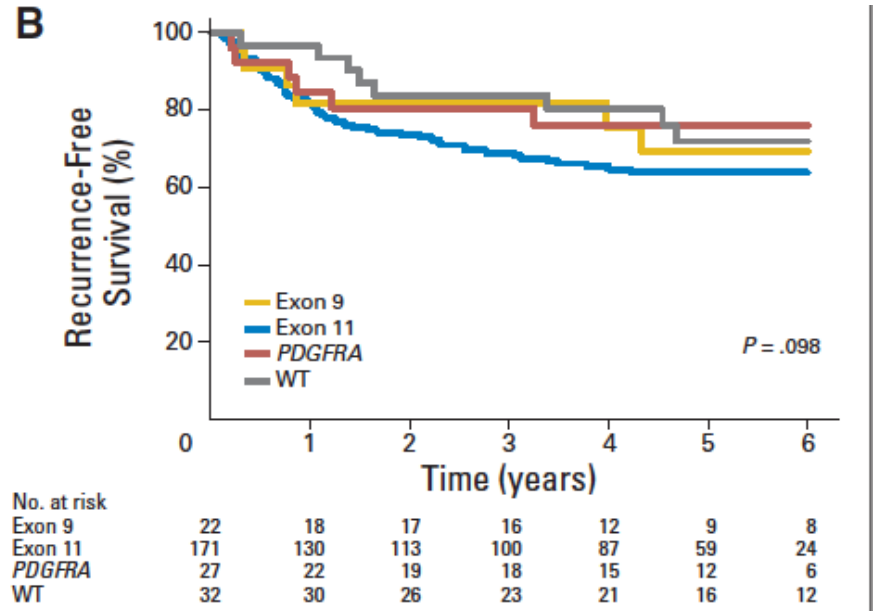
OS **+10%**

5-year survival, 92.0% vs 81.7%
HR, 0.45; 95% CI, 0.22 - 0.89
P=.02

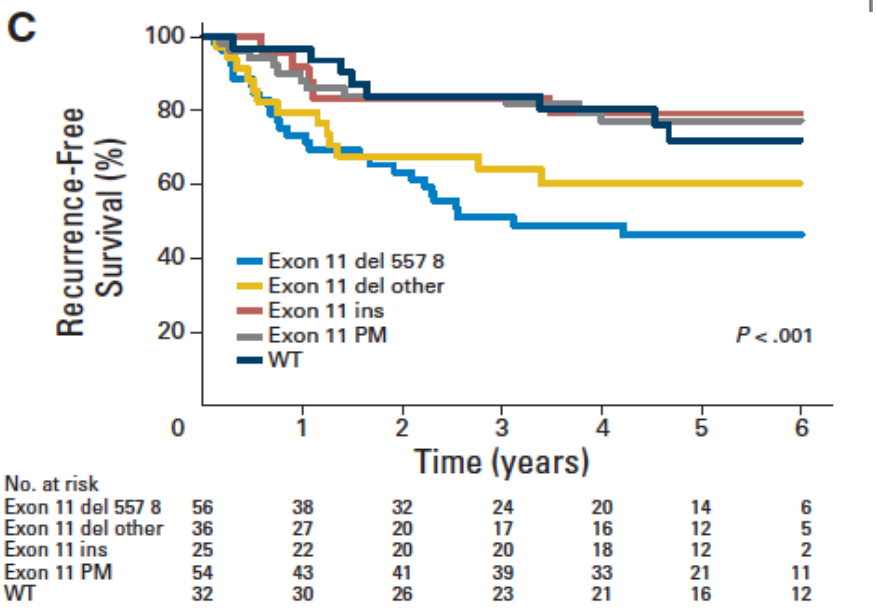
Joenssu et al JAMA 2012

Can GIST Genotype Refine Clinicopathologic Prognostication and / or Therapeutic Prediction?

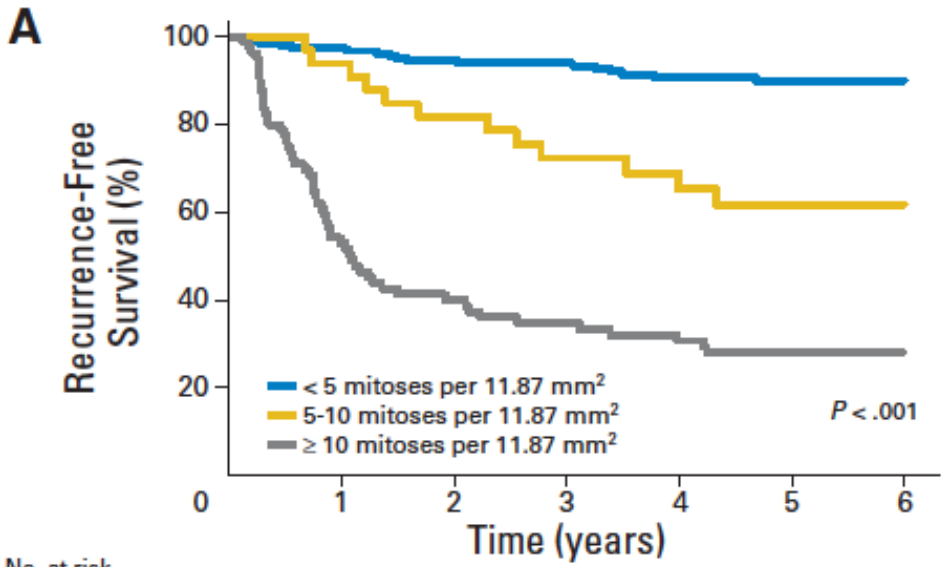
Data from placebo arm of randomised trial of adjuvant Imatinib in resected GIST (ACOSOG Z9001)



No. at risk							
Exon 9	22	18	17	16	12	9	8
Exon 11	171	130	113	100	87	59	24
PDGFRA	27	22	19	18	15	12	6
WT	32	30	26	23	21	16	12



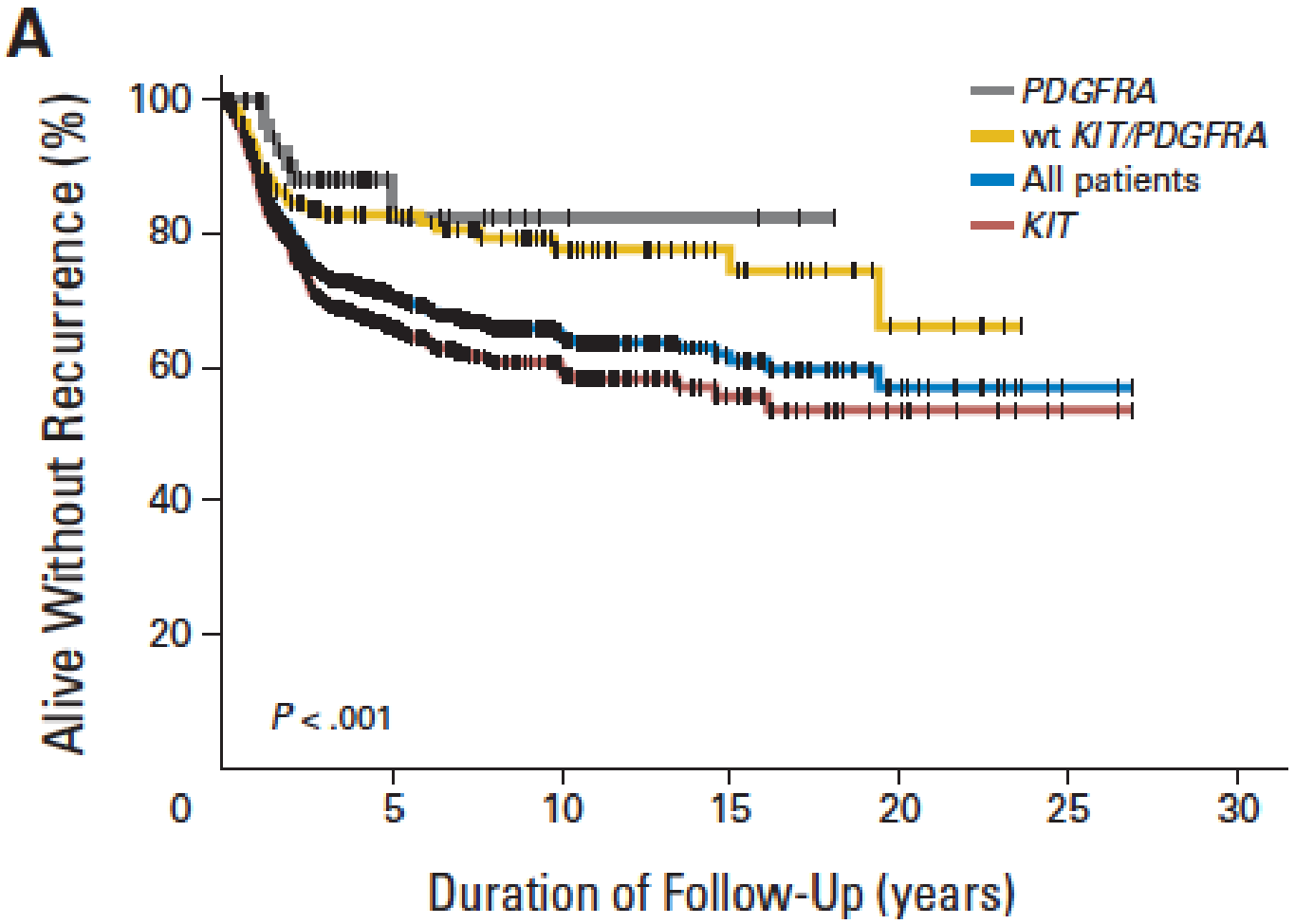
No. at risk							
Exon 11 del 557 8	56	38	32	24	20	14	6
Exon 11 del other	36	27	20	17	16	12	5
Exon 11 ins	25	22	20	20	18	12	2
Exon 11 PM	54	43	41	39	33	21	11
WT	32	30	26	23	21	16	12



No. at risk							
< 5 mitoses	199	180	167	152	131	92	46
5-10 mitoses	37	31	26	22	18	14	5
≥ 10 mitoses	81	41	31	26	23	15	7

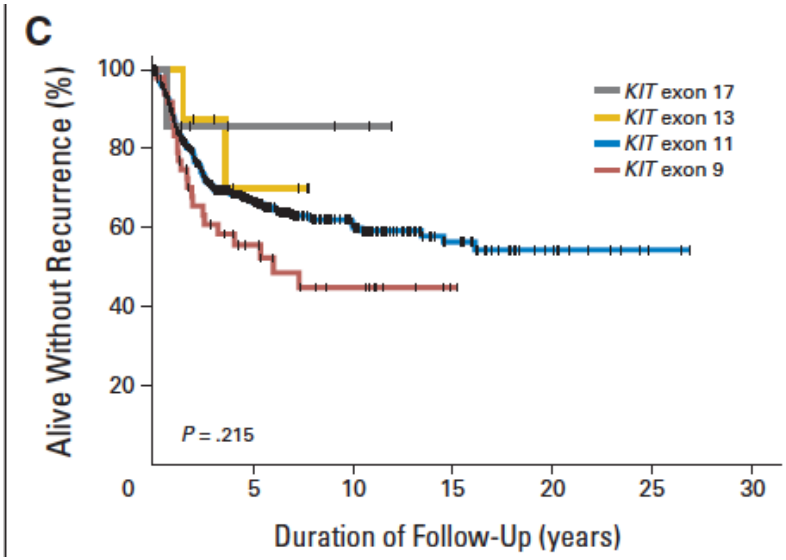
Corless et al JCO 2014

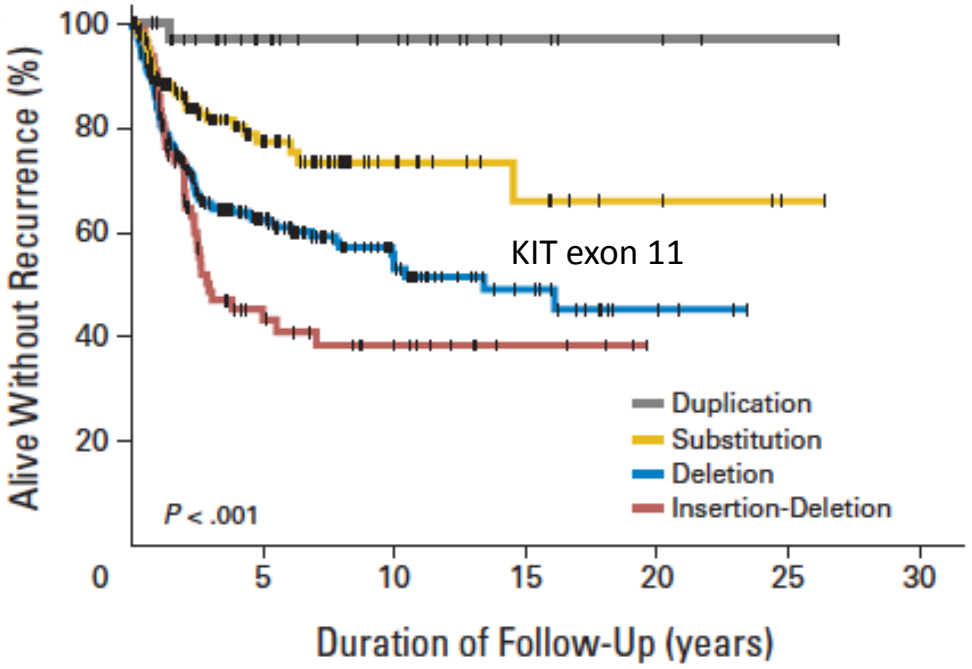
Data from pooled analysis of population based series (n=1505) of resected GIST w/o adjuvant Imatinib

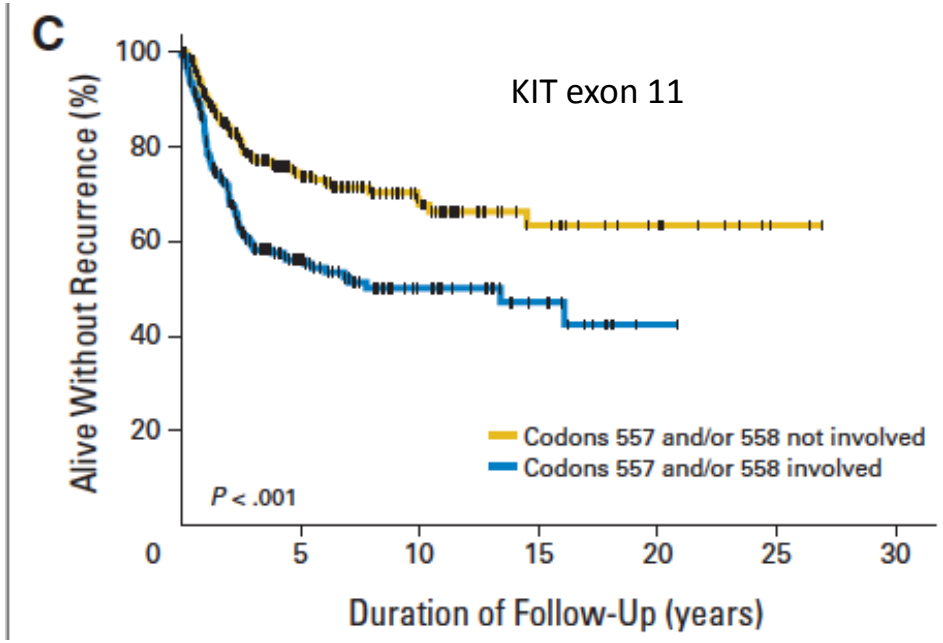


Joenssu et al JCO 2015

Prognostic Influence of Different KIT mutations







Joenssu et al JCO 2015

Relative Prognostic Influence of Classic Clinicopathological Factors

Patients with a generally unfavorable mutation, (e.g. exon 11 deletion mutation causing Try557_Lys558 deletion) were still at low risk for GIST recurrence, provided that the mitotic count was very low. In contrast, patients with PDGFRA mutations had a high risk of recurrence when tumor mitotic count was high.

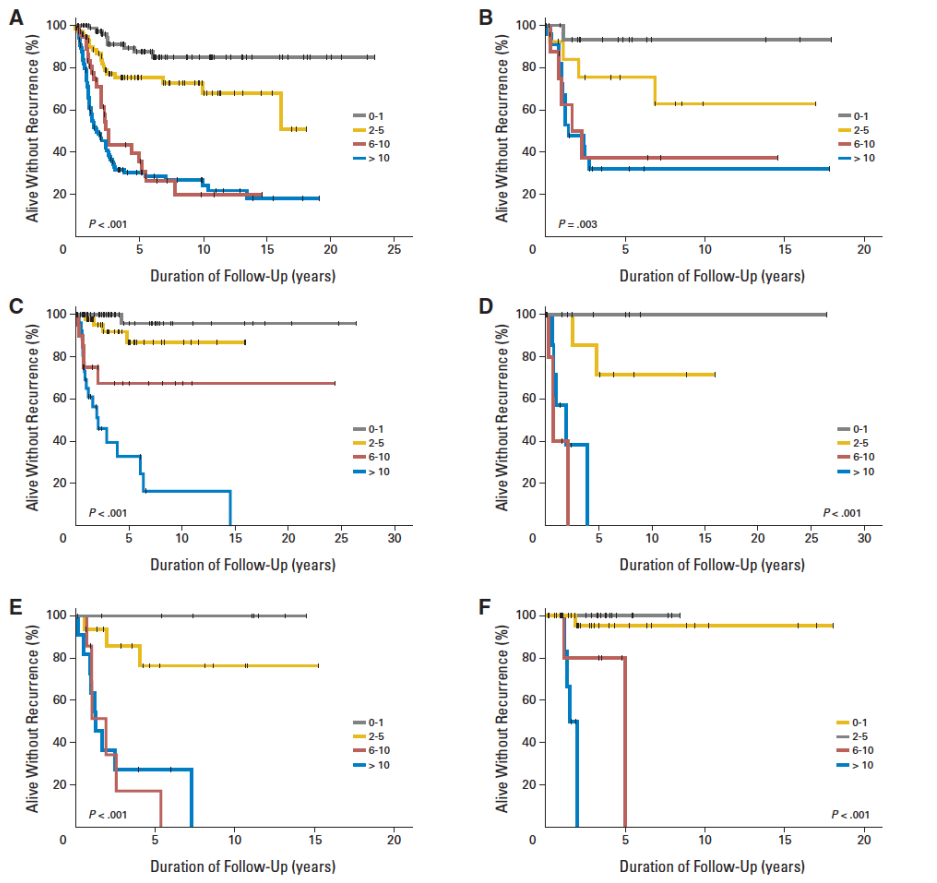


Table A4. Results of Cox Multivariable Hazards Model With Mutated *KIT* Exon, Tumor Size, Mitotic Count, and Tumor Site As Covariables

Covariable	Hazard Ratio	95% CI	*P*
Mitotic count per 50 HPFs (continuous)	1.018	1.014 to 1.021	< .001
Tumor size, cm (continuous)	1.109	1.080 to 1.138	< .001
Tumor site			< .001
GI tract, outside of the stomach	Reference		
E-GIST	0.644	0.247 to 1.678	.367
Stomach	0.398	0.282 to 0.563	< .001
Mutated *KIT* exon			.427
Exon 9	Reference		
Exon 11	1.224	0.739 to 2.026	.432
Exon 13	0.482	0.112 to 2.065	.325
Exon 17	0.526	0.070 to 3.942	.532

Joenssu et al JCO 2015

Amongst KIT/PDGFR mutants,

- Certain genotypes predict for decreased / nil Imatinib sensitivity

- In considering prognosis for resected localised GIST, clinicopathologic risk factors (especially mitotic count) retain superior prognostic value over genotype

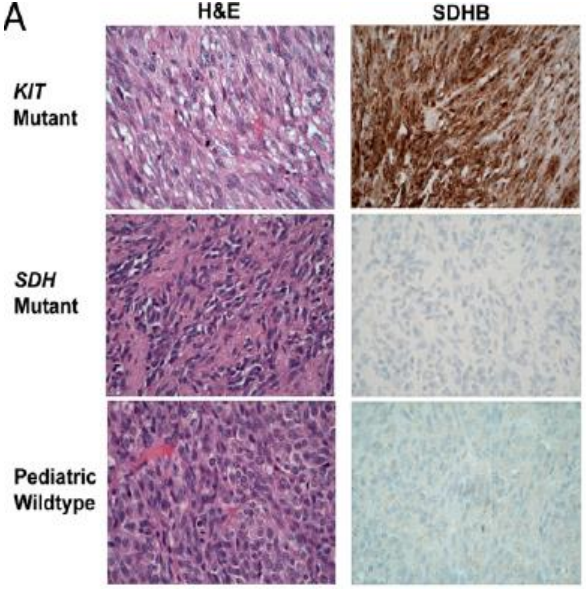
- KIT deletions / insertions-deletions have inferior prognosis compared with other types of KIT mutations

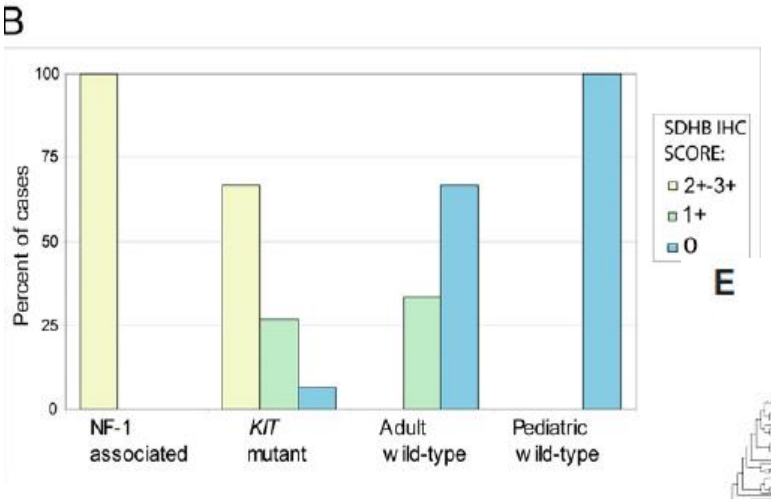
- Non-KIT mutants (PDGFR, non KIT non PDGFR) may have superior prognosis compared with KIT mutants

Non -KIT, Non-PDGFRA GIST (~10-%)

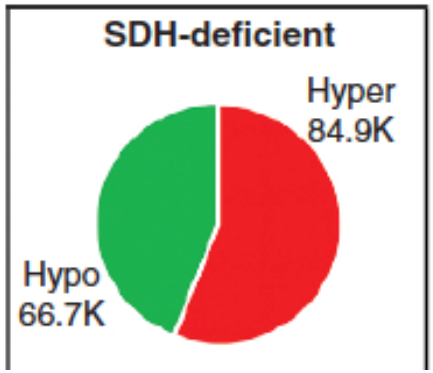
Genotype	Relative Frequency	Anatomic Distribution	Germline Examples
BRAFV600E	3%		None
NF1- related	<1%	Small bowel	Yes
HRAS, NRAS, PIK3CA	<1%		None
SDHA, SDHB, SDHC, SDHD mutations	6%	Stomach and small bowel	Yes (including Carney Stratakis syndrome)
SDHC hypermethylation – Carney triad	~1%	stomach	No
Quadruple WT	Rare		No

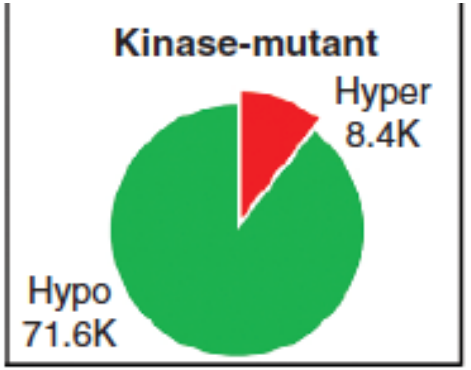
SDH Deficient GIST

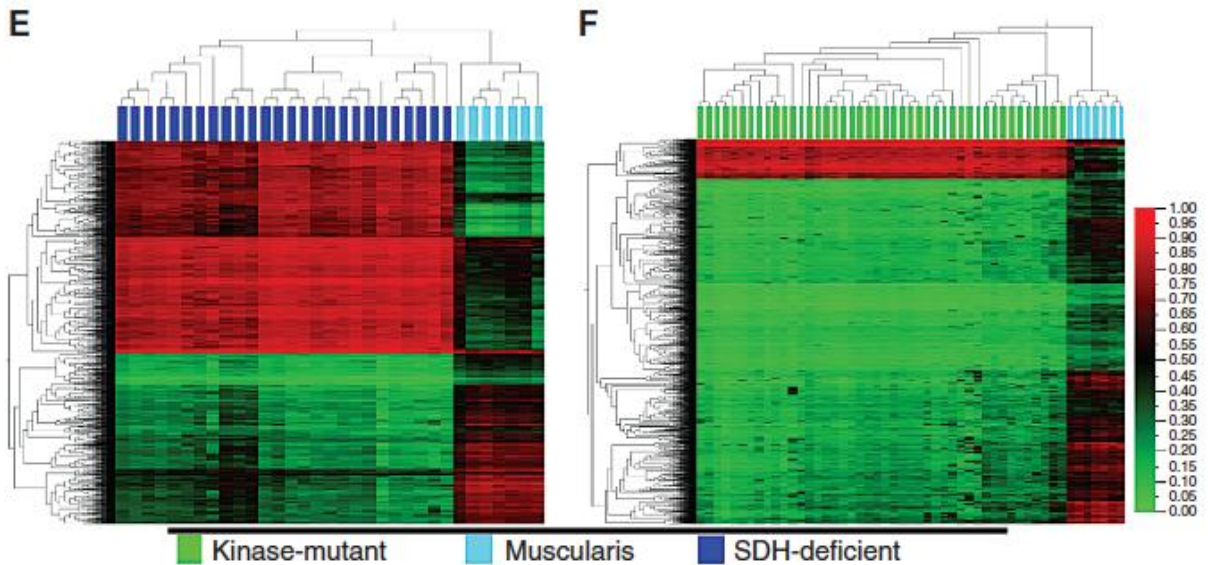




Janeway et al PNAS 2011

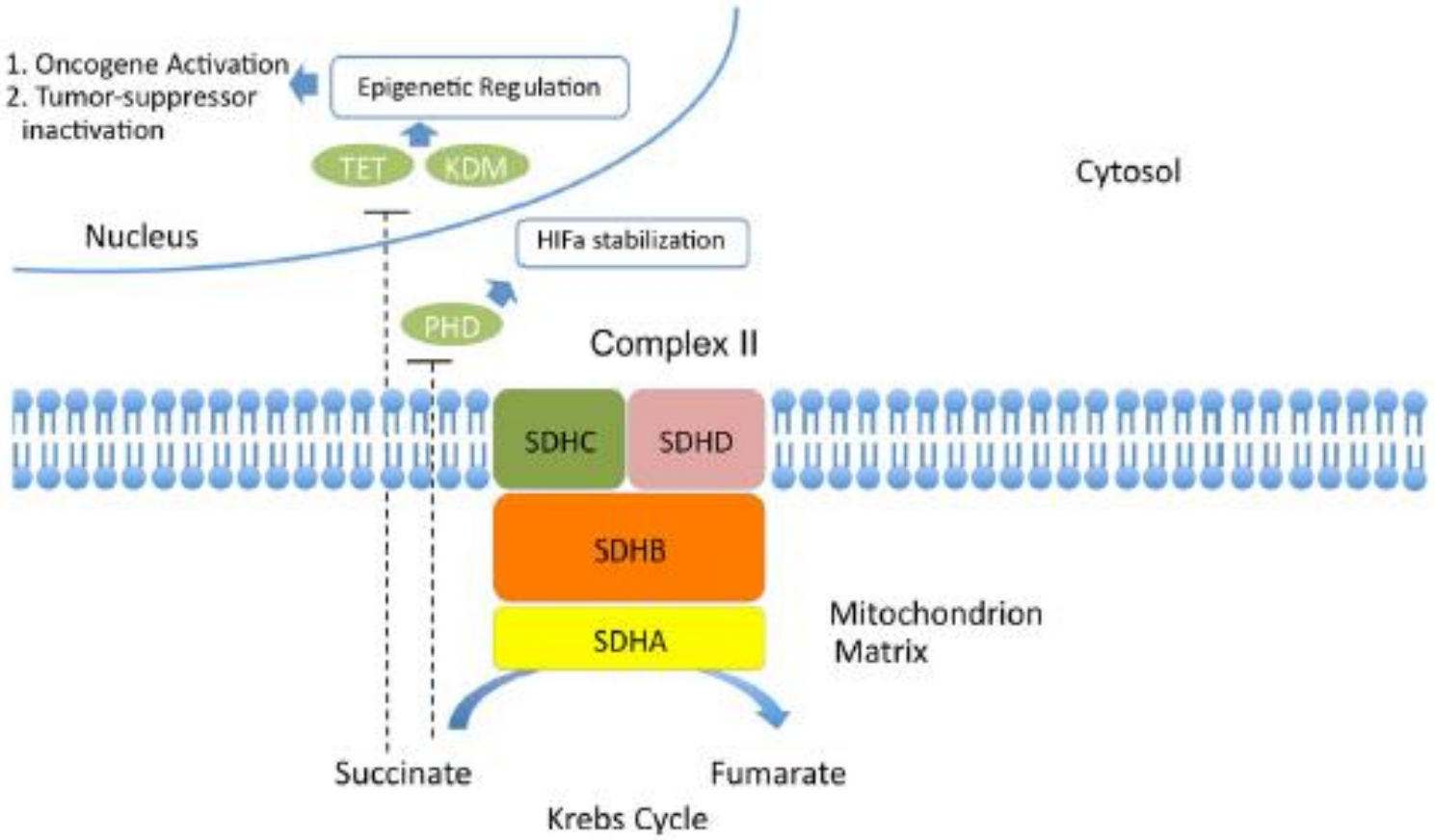




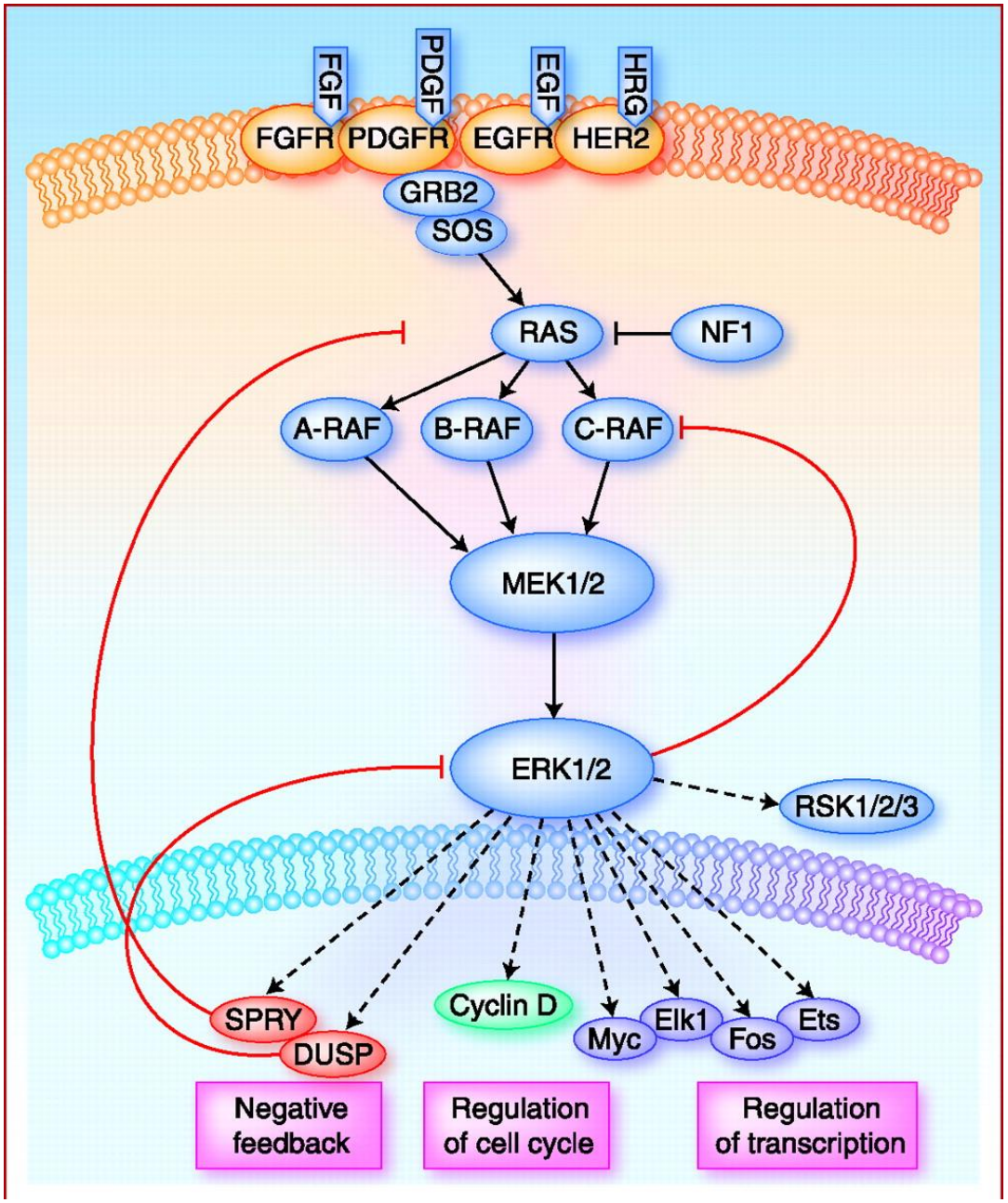


Killian et al Cancer Disc 2013

Succinate Accumulation and Oncogenesis



The MAPK Pathway



Pratilas et al CCR 2010

Non KIT, non PDGFR GIST – clinical aspects

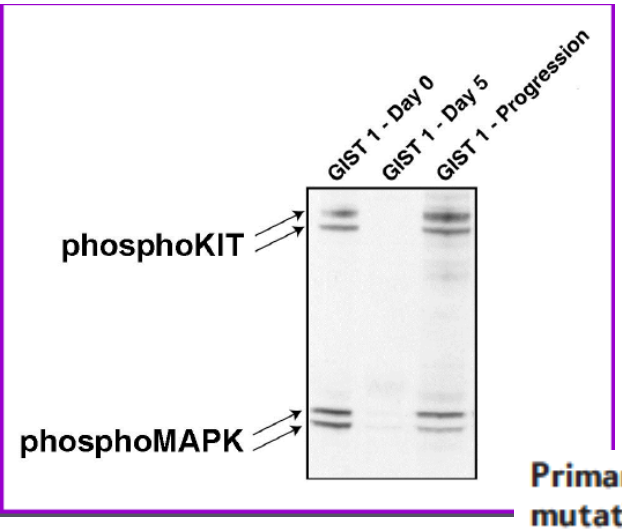
- Majority of pediatric GISTs (1-2% of all GISTs) are SDH-immunonegative

- Approximately 7% of NF1 patients develop GIST – can be multicentric and associated with ICC hyperplasia, as in familial GISTs with germline KIT/PDGFR mutations

- Even in large series, absolute numbers are small, but non-KIT, non PDGFR GISTs generally disposed to a more indolent biology, and less sensitive to Imatinib

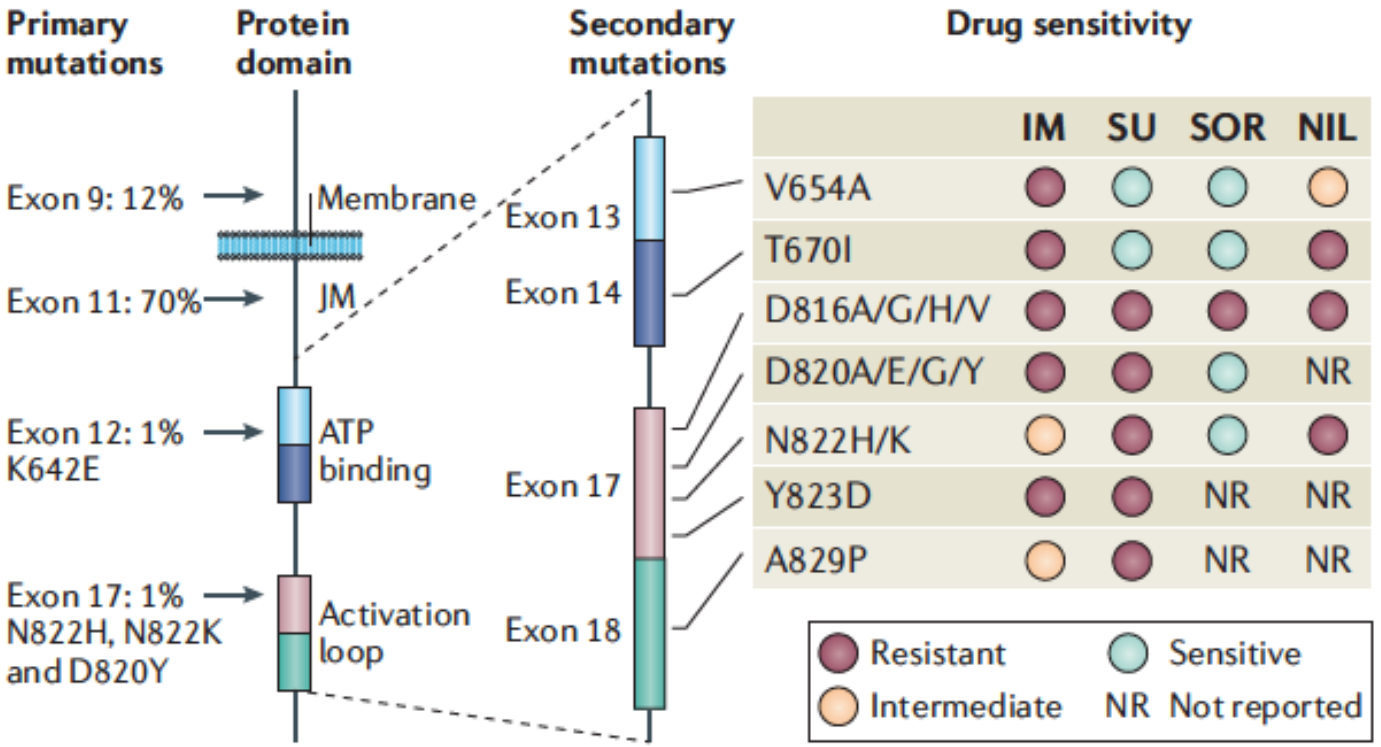
- This may have bearing on use of adjuvant Imatinib in these genotypes

Development of Imatinib Resistance



Fletcher et al 2012

50% of patients develop resistance within the first 2 years from intra-allelic secondary KIT mutations that abrogate Imatinib binding or activity.



Corless et al NRC 2011

Conclusions

- In spite of its relative cytogenetic and genomic simplicity and its status as the archetypal oncogene-addicted tumour, GIST represents a molecularly complex family of tumours rather than a uniform biological entity

- KIT is the gene most commonly mutated (80%) in primary disease
- KIT-mutant GISTs can have varying prognoses, but are generally Imatinib sensitive
- PDGFR mutant GISTs (10%) can be associated with superior prognosis compared with KIT-mutant GISTs; the PDGFRd842 mutation confers Imatinib resistance – these patients should not receive Imatinib.

Conclusions

- The 10% of GIST wild type for KIT and PDGFR demonstrate an evolving complexity, with the best characterized groups being the MAPK-pathway mutated and SDH deficient GISTs

- These GISTs are also generally predisposed to indolent biologies and relative Imatinib resistance (data from very small numbers).

- Classic clinicopathologic risk factors are more important that genotype in prognosticating resected localised GIST; however, genotype is important to consider in deciding on use of adjuvant therapy as some genotypes predict for Imatinib insensitivity.

- Imatinib resistant KIT-mutated GIST is almost always driven by secondary mutations in KIT, for which second line therapies have varying activities depending on the nature of the secondary mutation. The utility of genotype directed therapy in this setting is limited by clonal heterogeneity and ongoing evolution of the resistant tumours.

Back Up Slides

THANK YOU

<farid.h.r@nccs.com.sg>

MUTATION DETECTED

c.1679_1681delTTG(p.V560del,Exon 11 deletion)

Interpretation:

Deletion of 3 nucleotides at position c.1679_1681 resulting in a deletion in exon 11 of cKIT gene that removes amino acid codon 560 (p.Val560del).

CLINICAL INTERPRETATION:

Gastrointestinal Stromal Tumours (GISTs)
Exon 11 deletions and deletion-insertions comprise about 75% of known KIT mutations identified so far in gastrointestinal stromal tumours (GISTs), and mutations in exon 9 accounts for the majority of the remaining mutations.
Current data suggest that mutations in exon 11 are associated with improved response to tyrosine kinase inhibitor (TKI) imatinib. Current evidence also suggests that mutations in exon 9 may be associated with improved response rates with higher daily dose of imatinib.

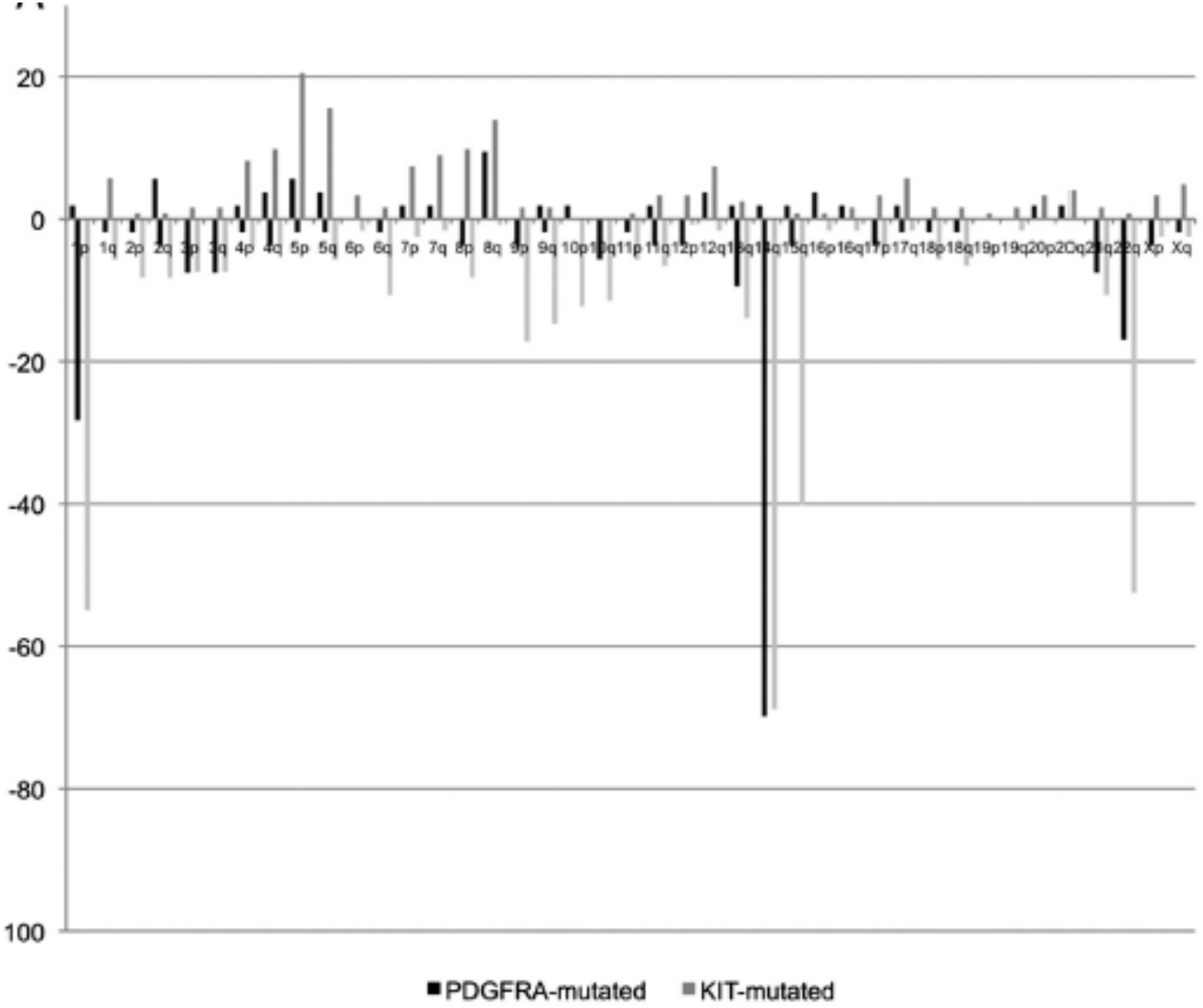
Melanomas
Activating KIT mutations are found in 15% of anal, 11-23% of acral, 15-21% of mucosal, and 16-27% of melanomas on sun-damaged skin. KIT mutations in melanoma are most frequently detected in exon 11 (61%), followed by exon 13 (25%), exon 17 (12%), and exon 18 (3%). Melanoma patients with KIT mutations have been shown to benefit from imatinib therapy in small studies.

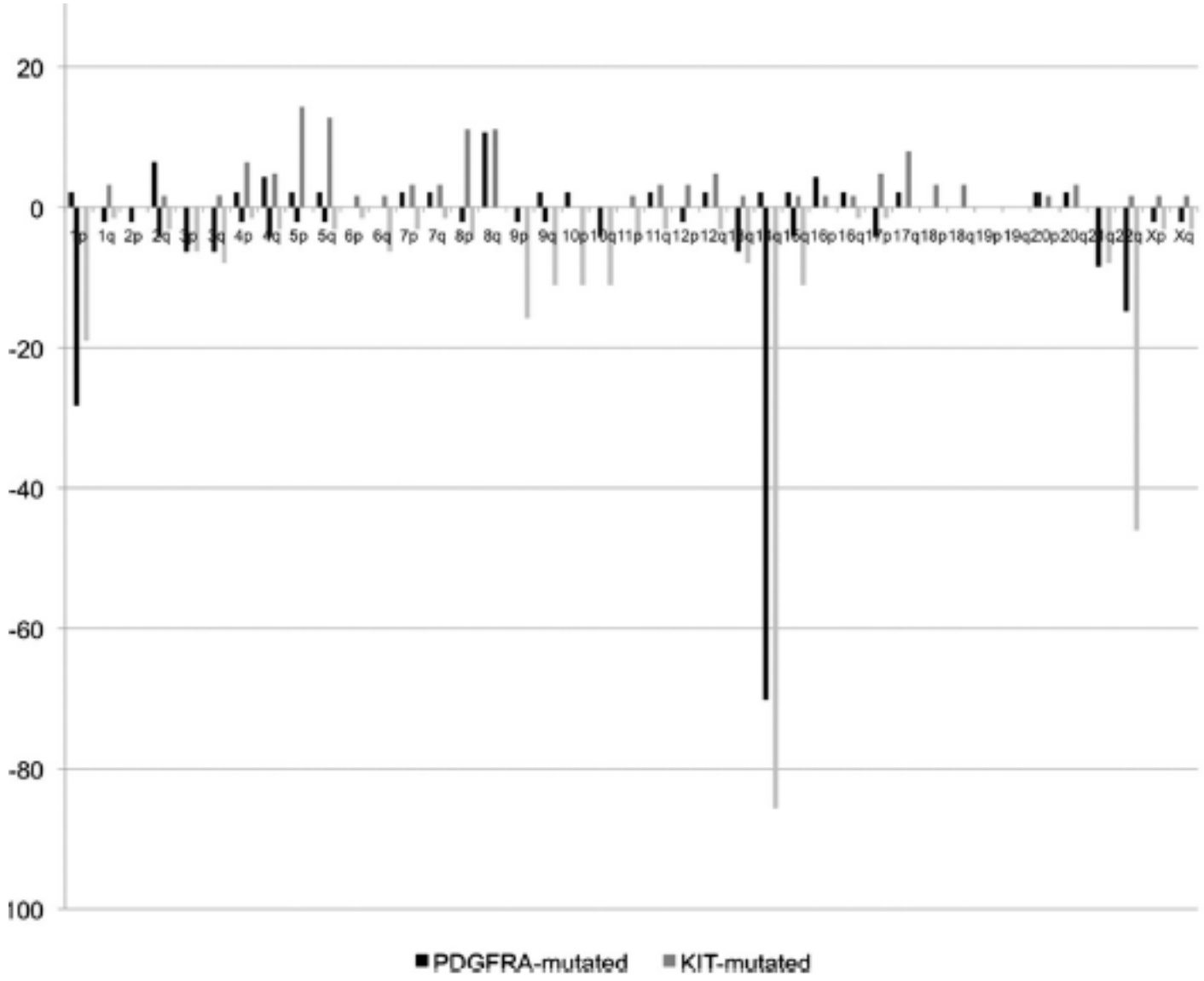
METHOD:
C-kit mutation analysis is carried out by a laboratory-developed direct Sanger sequencing assay. Genomic DNA is extracted from the tissue sample. The extracted DNA undergoes PCR amplification for exons 9 and 11, and the products are analysed by direct sequencing.

The following mutations are screened for in our assay:
Exon 9: p.A502_Y503dup
Exon 11: p.W557G, p.W557R, p.V559A, p.V559G, p.V560G, p.V560D, p.L576P,
deletions and complex deletion-insertions.

Mutations other than those listed above are not generally searched for. Our test validation demonstrated a lower limit of detection (LOD) of our assay at 25% mutated cells in a background of wild-type cells.

REFERENCES:
1.KIT mutations are common in incidental gastrointestinal stromal tumors one centimeter or less in size. Corless CL et al., Am J Pathol 2002; 160: 1567-1572.
2.Correlation of Kinase Genotype and Clinical Outcome in the North American Intergroup Phase III Trial of Imatinib Mesylate for Treatment of Advanced Gastrointestinal Stromal Tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. Heinrich et al., J Clin Oncol 2008; 26:5360-5367.
3.Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal. Tumours. Lasota J & Miettinen M (2008) Histopathology 53, 245-266.


20
0
-20
-40
-60
-80
100
1p 1q 2p 2q 3p 3q 4p 4q 5p 5q 6p 6q 7p 7q 8p 8q 9p 9q 10p 10q 11p 11q 12p 12q 13q 14q 15q 16p 16q 17p 17q 18p 18q 19p 19q 20p 20q 21q 22q Xp Xq
PDGFRA-mutated
KIT-mutated


20
0
-20
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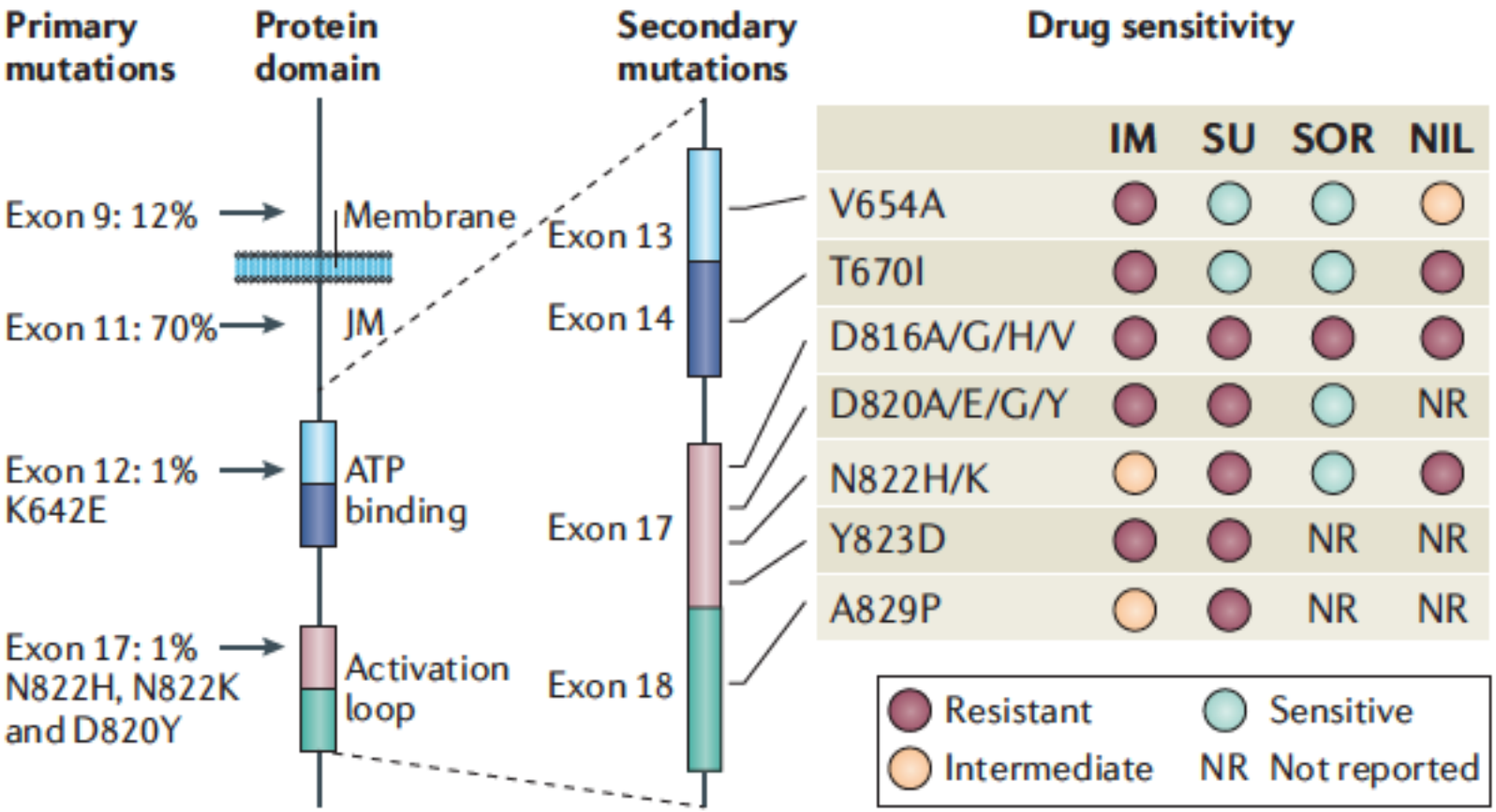
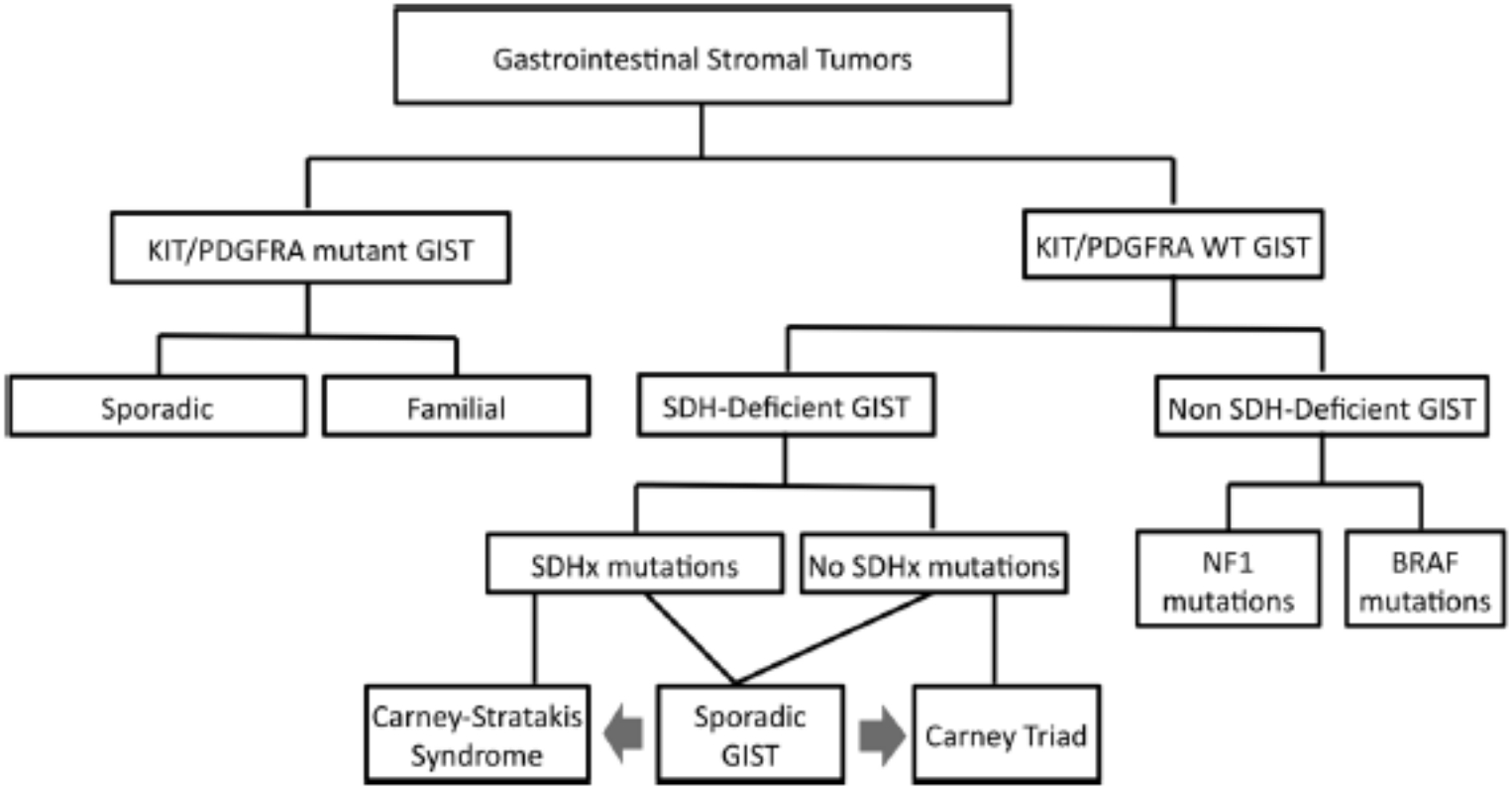
-60
-80
100
1p 1q 2p 2q 3p 3q 4p 4q 5p 5q 6p 6q 7p 7q 8p 8q 9p 9q 10p 10q 11p 11q 12p 12q 16q 16p 16q 17p 17q 18p 18q 19p 19q 20p 20q Xp Xq
PDGFRA-mutated
KIT-mutated


Primary mutations
Protein domain
Secondary mutations
Drug sensitivity
Exon 9: 12%
Membrane
Exon 11: 70%
JM
Exon 12: 1%
K642E
ATP binding
Exon 17: 1%
N822H, N822K
and D820Y
Activation loop
Exon 13
Exon 14
Exon 17
Exon 18
IM
SU
SOR
NIL
V654A
T670I
D816A/G/H/V
D820A/E/G/Y
N822H/K
Y823D
A829P
NR
NR
NR
NR
NR
NR
Resistant
Intermediate
Sensitive
NR Not reported

Genetic type	*Relative frequency (%)*	*Anatomic distribution*	*Germline example*
KIT mutation	75		
Exon 8	Rare	Small bowel	One kindred
Exon 9 ins AY502–503	8	Small bowel, colon	None
Exon 11 (deletions, single nucleotide substitutions, and insertions)	65	All sites	Several kindreds
Exon 13 K642E	1	All sites	Three indreds
Exon 17 D820Y, N822K, and Y823D	1	All sites	Five kindreds
PDGFRA mutation	10		
Exon 12 (eg, V561D)	1	All sites	Two kindreds
Exon 14 N659K	Rare	Stomach	None
Exon 18 D842V	6	Stomach, mesentery, omentum	None
Exon 18 (eg, del IMHD 842-846)	2	All sites	One kindred
KIT and *PDGFRA* wild type	15	All sites	
BRAF V600E	~2		None
SDHA/B/C/D mutations	~6	Stomach and small bowel	Carney–Stratakis
HRAS, *NRAS*, and *PIK3CA* mutation	<1		None
Pediatric/Carney triad	~1	Stomach	Not heritable
NF1-related	<1	Small bowel	Numerous


Gastrointestinal Stromal Tumors
KIT/PDGFRA mutant GIST
KIT/PDGFRA WT GIST
Sporadic
Familial
SDH-Deficient GIST
Non SDH-Deficient GIST
SDHx mutations
No SDHx mutations
NF1 mutations
BRAF mutations
Carney-Stratakis Syndrome
Sporadic GIST
Carney Triad

Genetic type	Relative frequency	Anatomic distribution	Germline examples
KIT mutation (relative frequency 75–80%)			
Exon 8	Rare	Small bowel	One kindred
Exon 9 insertion AY502-503	10%	Small bowel and colon	None
Exon 11 (deletions, single nucleotide substitutions and insertions)	67%	All sites	Several kindreds
Exon 13 K642E	1%	All sites	Two kindreds
Exon 17 D820Y, N822K and Y823D	1%	All sites	Five kindreds
PDGFRA mutation (relative frequency 5–8%)			
Exon 12 (such as V561D)	1%	All sites	Two kindreds
Exon 14 N659K	<1%	Stomach	None
Exon 18 D842V	5%	Stomach, mesentery and omentum	None
Exon 18 (such as deletion of amino acids IMHD 842–846)	1%	All sites	One kindred
KIT and PDGFRA wild-type (relative frequency 12–15%)			
BRAF V600E	~7–15%		
SDHA, *SDHB*, *SDHC* and *SDHD* mutations	~2%	Stomach and small bowel	Carney–Stratakis
HRAS and *NRAS* mutation	<1%		
Sporadic paediatric GISTs	~1%	Stomach	Not heritable
GISTs as part of the Carney triad	~1%	Stomach	Not heritable
NF1-related	Rare	Small bowel	Numerous

Table 1. Associations Between the Type of *KIT* Exon 11 Mutation and GIST Site, Size, and Mitotic Count

Type of *KIT* Exon 11 Mutation	Total Patients		Different Mutations		GIST Site										Tumor Size (cm)		Mitotic Count (per 50 HPFs)	
					Esophagus		Stomach		Small Intestine		Colon or Rectum		Non-GI Tract*		Site NA			
	No. of Patients	%	No.	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	(No.)	Median	Range	Median	Range
Deletion	430	43.8	111	37.9	5	55.6	254	44.2	132	42.7	18	45.0	15	42.9	6	6.0	0.1-33.0	5	0-250
1 codon	71	7.2	9	3.1	1	11.1	45	7.8	21	6.8	2	5.0	2	5.7	0	5.2	0.9-22.0	3	0-60
≥ 2 codons	359	36.6	102	34.8	4	44.4	209	36.3	111	35.9	16	40.0	13	37.1	6	7.0	0.1-33.0	5	0-250
Insertion-deletion	155	15.8	96	32.7	3	33.3	73	12.7	60	19.4	15	37.5	2	5.7	2	6.4	0.7-26.0	5	0-250
Duplication	52	5.3	37	12.6	0	0	45	7.8	7	2.3	0	0	0	0	0	4.5	1.5-19.0	3	0-45
Insertion	27	2.8	13	4.4	1	11.1	16	2.8	4	1.3	0	0	3	8.6	3	8.0	0.5-45.0	4	0-34
Substitution	300	30.6	19	6.5	0	0	176	30.6	103	33.3	7	17.5	12	34.3	2	5.5	0.3-30.0	3	0-150
Other†	17	1.7	17	5.8	0	0	11	1.9	3	1.0	0	0	3	8.6	0	4.5	0.6-20.0	2.5	0-13
Total	981	100.0	293	100.0	9	100.0	575	100.0	309	100.0	40	100.0	35	100.0	13	6.0	0.1-45.0	4	0-250

Table A7. Risk of GIST Recurrence According to Prognostic Heat Maps in Patient Populations With Prognostically Favorable *KIT* or *PDGFRA* Mutations

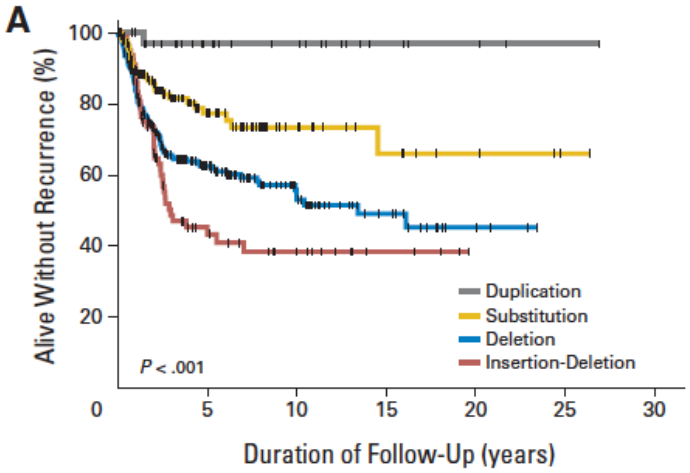
Risk for GIST Recurrence Within the First 10 Years After Surgery (%)	*KIT* Exon 11, Duplication Mutation (5-year RFS, 96.9%)		*KIT* Exon 11, Deletion of 1 Codon (5-year RFS, 82.2%)		*KIT* Exon 11 Mutation, Leading to Trp557Arg, Val559Ala, or Leu576Pro (5-year RFS, 82.5 to 95.2%)		*PDGFRA* Mutation (any; 5-year RFS, 82.2%)		Total	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
0-10	9	25.0	12	27.9	18	33.3	26	34.2	65	31.1
11-20	21	58.3	13	30.2	15	27.8	23	30.3	72	34.4
21-40	3	8.3	10	23.3	9	16.7	15	19.7	37	17.7
41-60	3	8.3	1	2.3	3	5.6	4	5.3	11	5.3
61-80	0	0	3	7.0	5	9.3	4	5.3	12	5.7
81-90	0	0	2	4.7	3	5.6	1	1.3	6	2.9
91-100	0	0	2	4.7	1	1.9	3	3.9	6	2.9

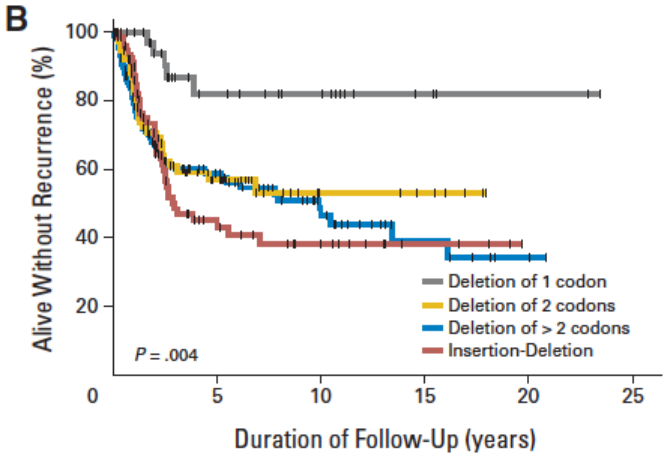
Genotype	Relative frequency	Germline examples
KIT mutation (relative frequency (70–80%)		
Exon 8	Rare	Yes
Exon 9 insertion AY502–503	10%	None
Exon 11 (deletions, single nucleotide substitutions, and insertions)	67%	Yes
Exon 13 K642E	1%	Yes
Exon 17 D820Y, N822K, and Y823D	1%	Yes
PDGFRA mutation (relative frequency 5–15%)		
Exon 12	1%	Yes
Exon 14	<1%	None
Exon 18 D842V	5%	None
Exon 18 (such as deletion of amino acids IMHD 842-846)	1%	Yes
KIT and *PDGFRA* wild-type (relative frequency 12–15%)		
BRAF V600E	3%	None
SDHA, *SDHB*, *SDHC*, and *SDHD* mutations	3%	Yes including Carney–Stratakis syndrome
SDHC hypermethylation—Carney triad	Rare	No
NF1-related	Rare	Yes
Quadruple wild-type	Rare	No

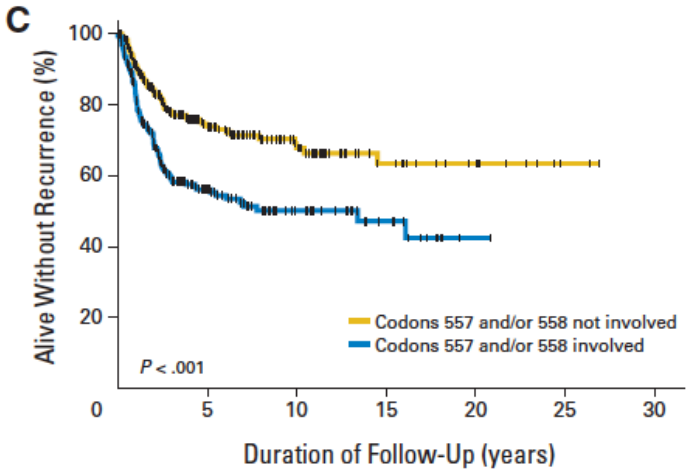
Genetic Type	Relative Frequency (%)	Anatomic Distribution	Notable Features
KIT mutation	77	—	—
Exon 8	Rare	Small bowel	
Exon 9	8	Small bowel, colon	Better responses higher-dose imatinib
Exon 11	67	All sites	Respond well to imatinib
Exon 13	1	All sites	Imatinib responsive
Exon 17	1	All sites	Many are imatinib sensitive
PDGFRA mutation	10	—	—
Exon 12	1	All sites	Sensitive to imatinib
Exon 14	<1	Stomach	Sensitive to imatinib
Exon 18 D842V	5	Stomach, mesentery, omentum	Imatinib resistant
Exon 18 other	1	All sites	Some but not all are imatinib sensitive
RTK-WT	13	All sites	—
RTK-WT/SDHB negative	—	—	—
SDH mutation (A/B/C/D)	~2	Stomach, small bowel	Carney-Stratakis syndrome
Carney triad	Rare	Stomach	Not heritable
Other (SDHA/B/C/D WT)	50–70 of pediatric GIST but <2 GIST	Stomach only	Most pediatric and adults <age 30–40 y
RTK-WT/SDHB positive	—	—	—
BRAF V600E mutation	~2	All sites	—
RAS mutations	<1	Stomach	—
NF1-related	~1	Small bowel	Multiple lesions, rarely malignant
Other	5–10	All sites	Most RTK-WT GIST in adults >30 y old

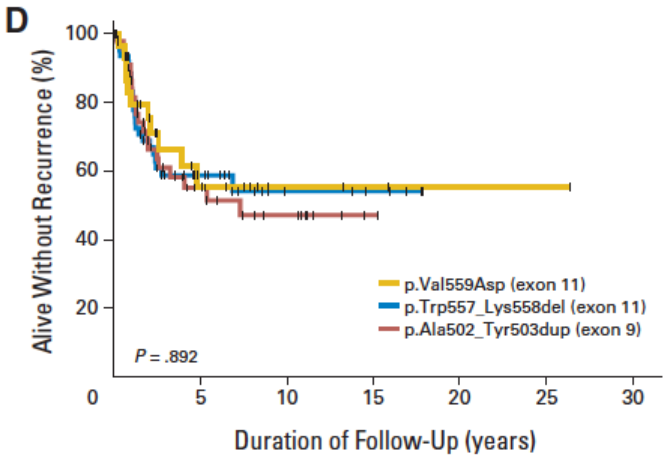
Modified NIH consensus criteria			
Very low risk	<2.0	≤5	Any site
Low risk	2.1–5.0	≤5	Any site
Intermediate risk	≤5.0	6–10	Gastric
	5.1–10.0	≤5	Gastric
High risk	>10.0	Any count	Any site
	Any size	>10	Any site
	>5.0	>5	Any site
	≤5.0	>5	Nongastric
	5.1–10.0	≤5	Nongastric
	Any size, site, or mitotic count if tumor rupture present		

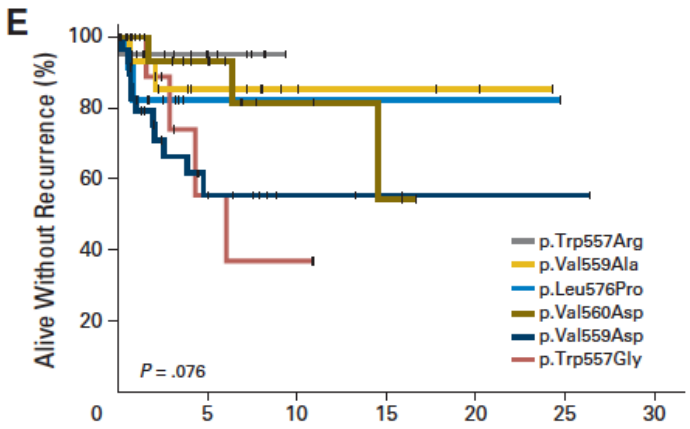
Risk Group	Group Size (%)	Time from Surgery			
		5-y RFS (%)	10-y RFS (%)	15-y RFS (%)	20-y RFS (%)
Modified NIH scheme					
Very low	11.9	97	95	95	95
Low	28.7	91	90	90	90
Intermediate	13.5	91	87	87	87
High	45.8	46	36	32	25

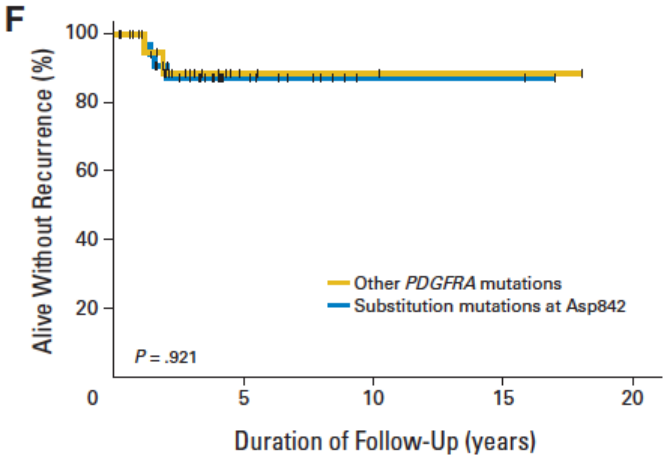

A
Alive Without Recurrence (%)
100
80
60
40
20
0
5
10
15
20
25
30
Duration of Follow-Up (years)
Duplication
Substitution
Deletion
Insertion-Deletion
P < .001

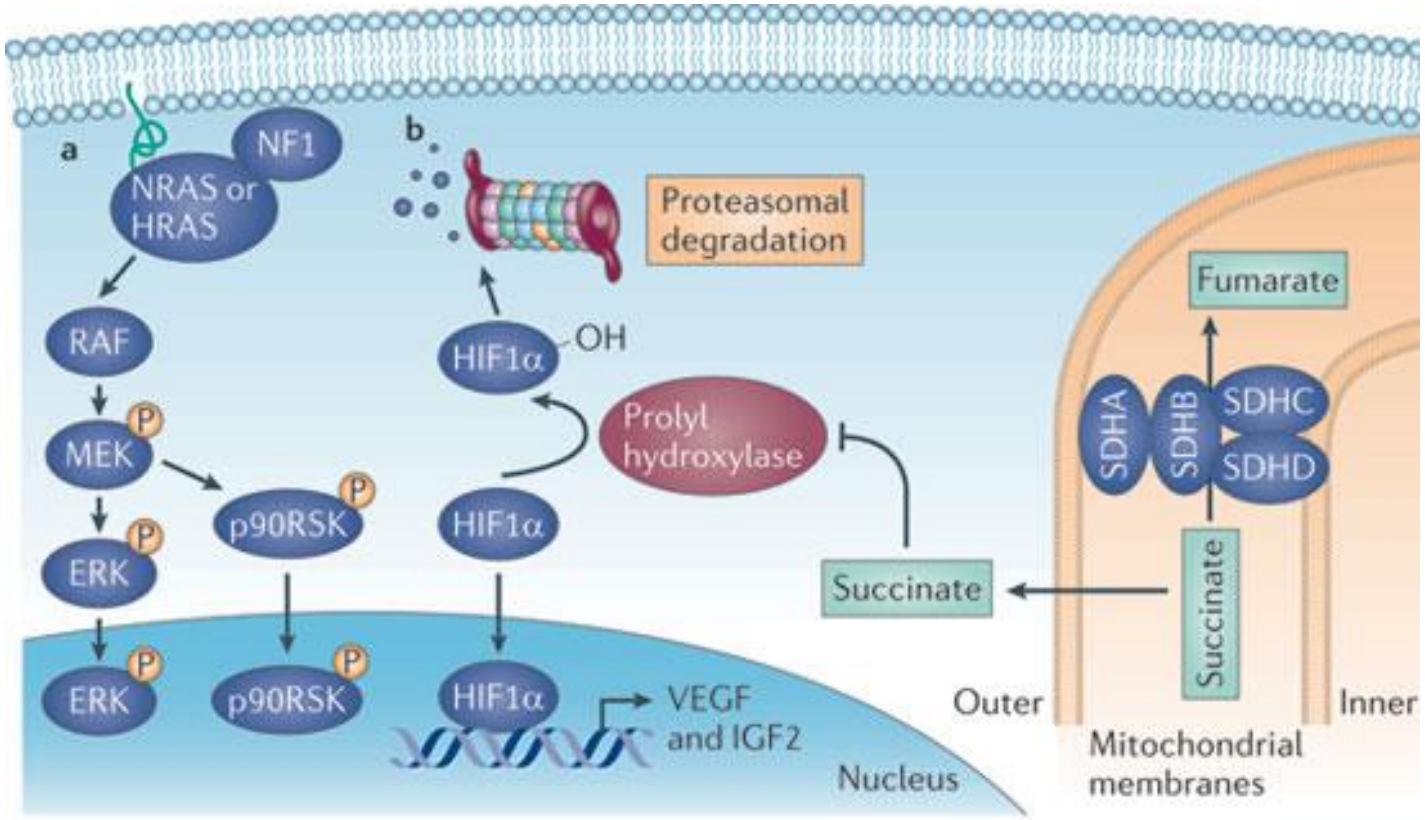

B
Alive Without Recurrence (%)
100
80
60
40
20
0
5
10
15
20
25
Duration of Follow-Up (years)
Deletion of 1 codon
Deletion of 2 codons
Deletion of > 2 codons
Insertion-Deletion
P = .004


C
Alive Without Recurrence (%)
100
80
60
40
20
0
5
10
15
20
25
30
Duration of Follow-Up (years)
Codons 557 and/or 558 not involved
Codons 557 and/or 558 involved
P < .001


D
Alive Without Recurrence (%)
100
80
60
40
20
0
5
10
15
20
25
30
Duration of Follow-Up (years)
p.Val559Asp (exon 11)
p.Trp557_Lys558del (exon 11)
p.Ala502_Tyr503dup (exon 9)
P = .892


E
Alive Without Recurrence (%)
100
80
60
40
20
0
5
10
15
20
25
30
p.Trp557Arg
p.Val559Ala
p.Leu576Pro
p.Val560Asp
p.Val559Asp
p.Trp557Gly
P = .076


F
Alive Without Recurrence (%)
100
80
60
40
20
0
5
10
15
20
Duration of Follow-Up (years)
Other PDGFRA mutations
Substitution mutations at Asp842
P = .921



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