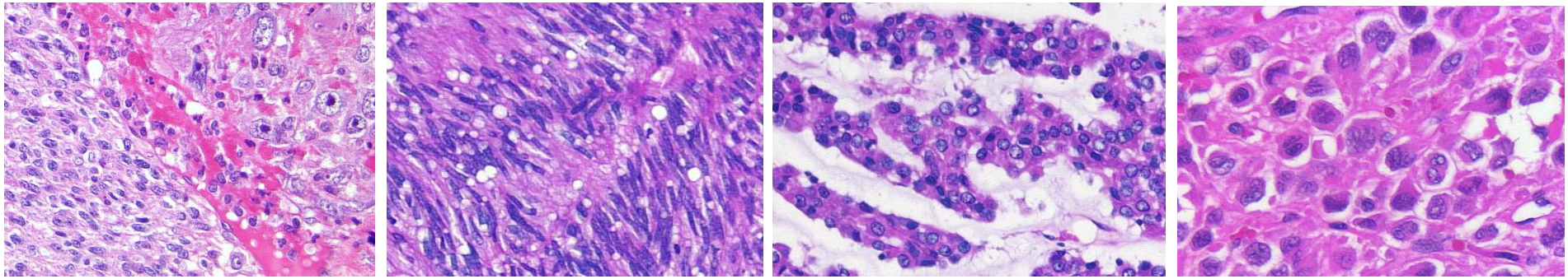
ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

SARCOMA & GIST CONFERENCE 2016



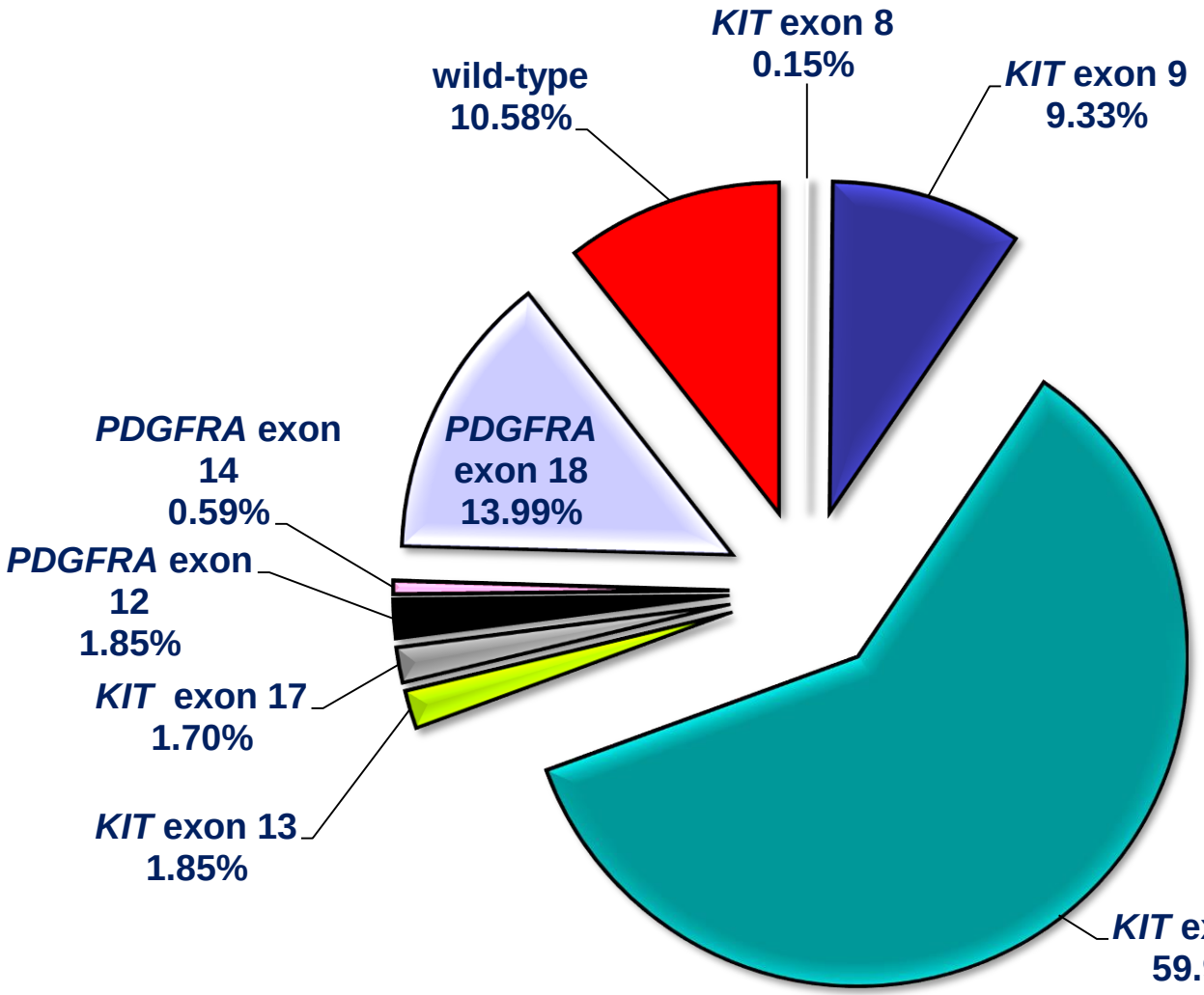
MOLECULAR GENETICS OF GIST

EVA WARDELMANN, GERHARD-DOMAGK-INSTITUTE OF PATHOLOGY, UNIVERSITY HOSPITAL MÜNSTER

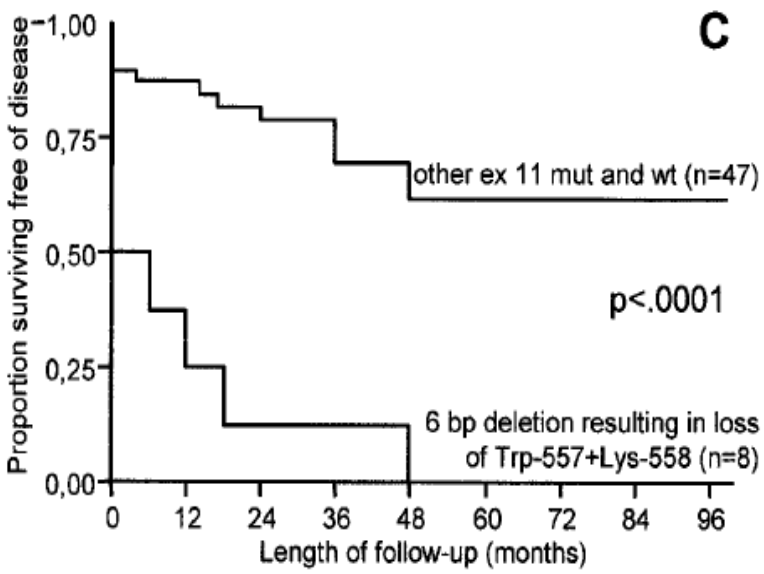
DISCLOSURE SLIDE

I have received honoraria and grants from Novartis, Pfizer, New Oncology, Ariad and PharmaMar

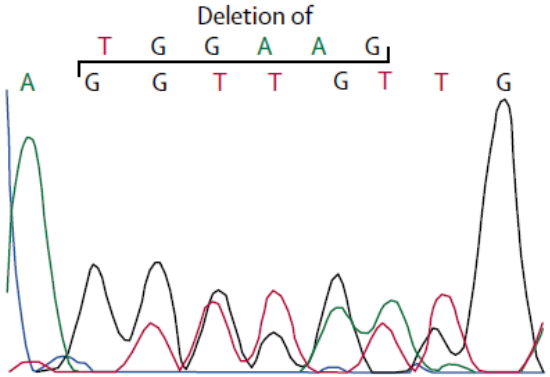
KIT and *PDGFRA* mutations occur in 85% to 90% of GIST (n=1351)



Prognostic relevance of deletions in *KIT*



Wardelmann, E et. al. Int J Cancer 2003; 106:887-895



KIT exon 11 6 bp deletion
(p.W557_K558del)

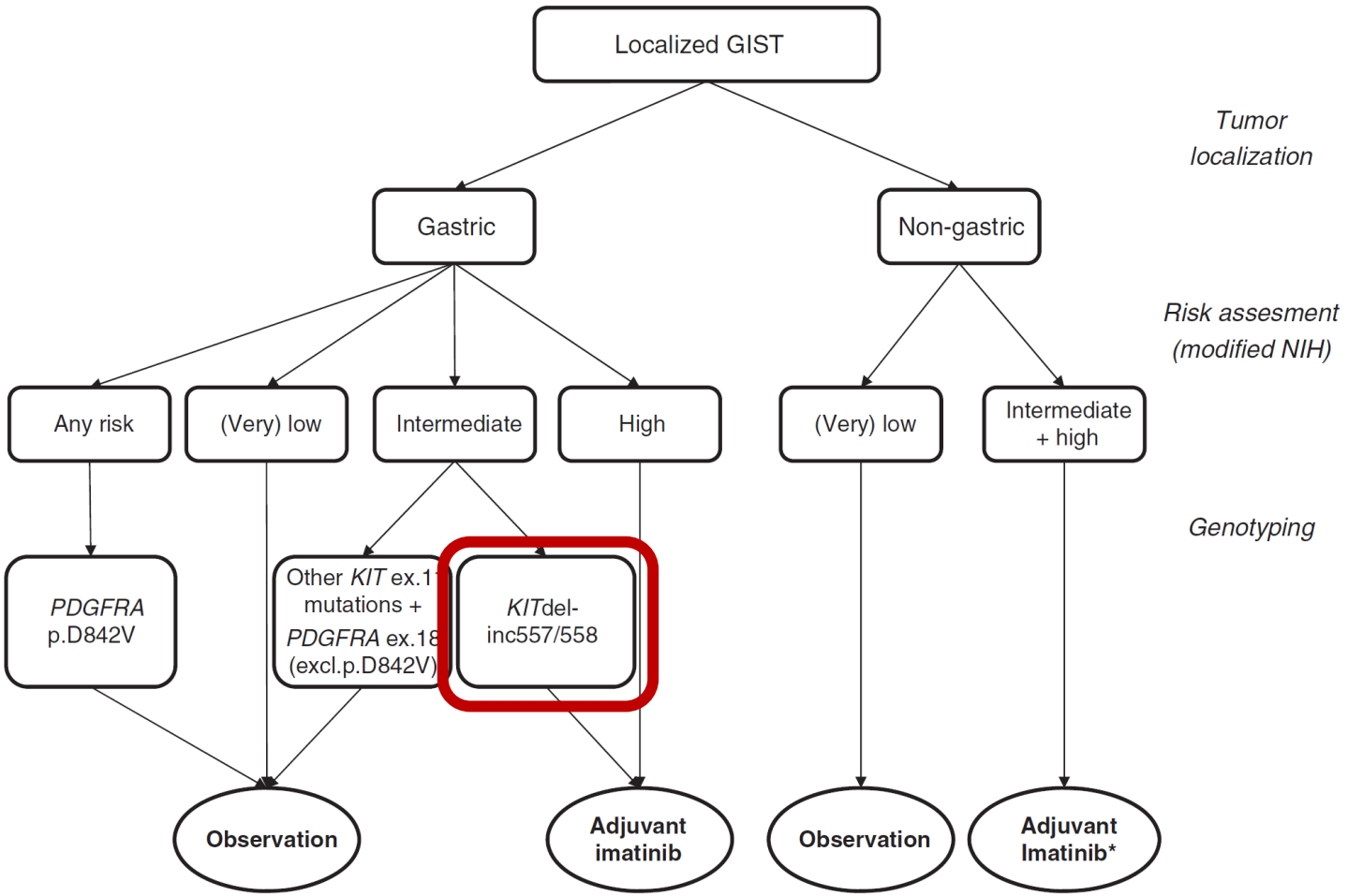
Martin J et al. J Clin Oncol 2005; 23:6190-6198

Martin-Broto et al., Ann Oncol 2010, 21:1552-1557

Wozniak A, Contica-GIST et al.; Clin Cancer Res 2014; 20:6105-6116

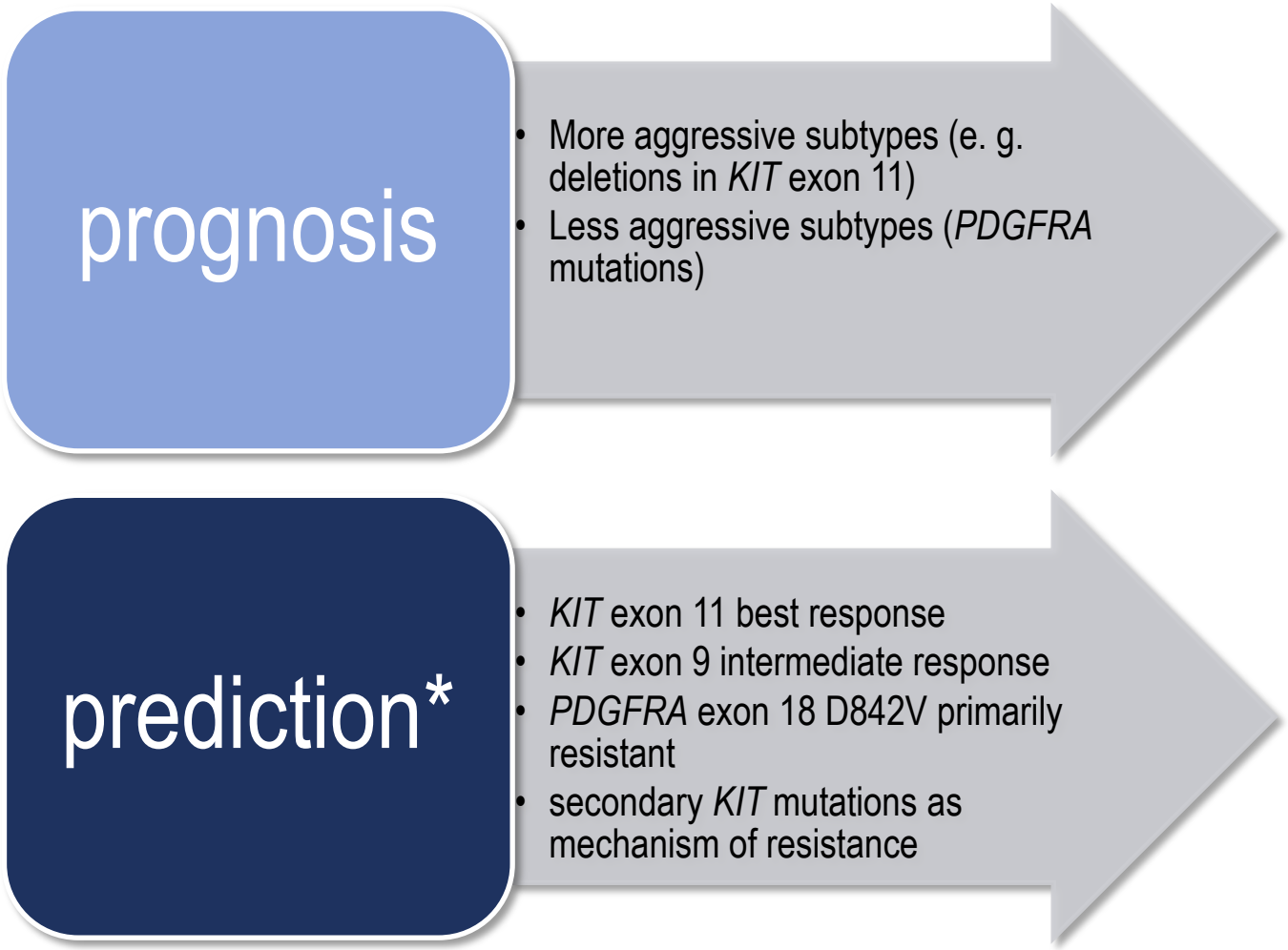
and others.....

Tumor Genotype Is an Independent Prognostic Factor in Primary Gastrointestinal Stromal Tumors of Gastric Origin: A European Multicenter Analysis Based on ConticaGIST



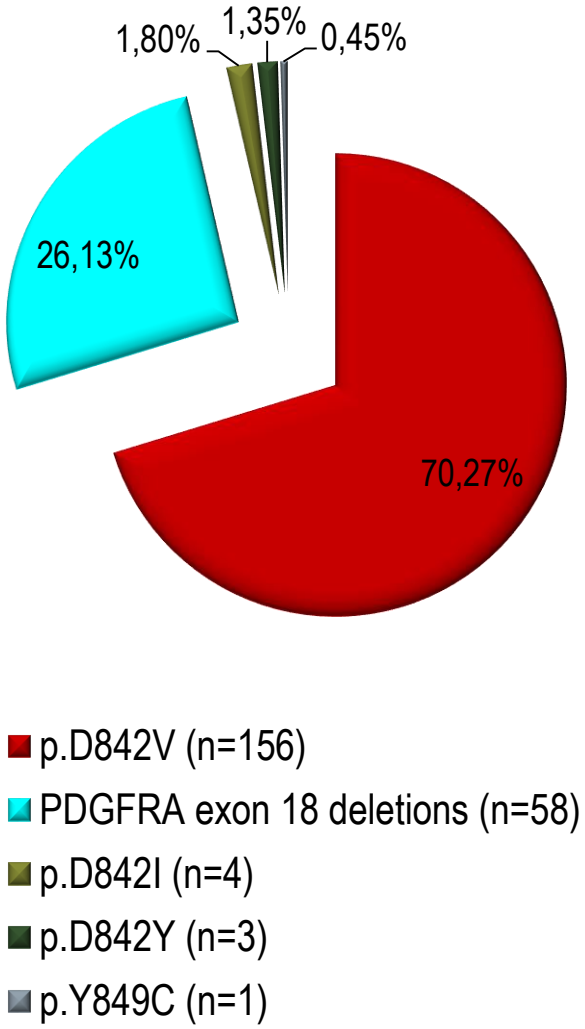
* Metastatic/locally advanced GIST with *KIT* ex. 9 mutations respond better to 800 mg imatinib daily (compared with the standard 400 mg). Therefore, increased dose may be considered in the adjuvant setting.

The mutational subtype is relevant for response prediction in GIST



*of response to treatment with imatinib

Frequency of resistance mutation p.D842V



- 15% of all GIST carry a *PDGFRA* exon 18 mutation
- nearly all are located in the stomach

~70% of all gastric *PDGFRA* exon 18 mutations are D842V!

10% of all GIST carry the resistance mutation D842V!

Lessions learned from hereditary GIST

- about 50 kindreds with germline *KIT* mutations are known world-wide
- identification of the underlying mutation may point towards novel hot spots
- identification of associated diseases can help to explain their pathogenesis
- three families have been identified so far in our German Registry

KIT Exon 17

Familial Gastrointestinal Stromal Tumors Caused by the Novel *KIT* Exon 17 Germline Mutation N822Y

Andreas Thalheimer, MD, Marcus Schlemmer, MD,† Marco Bueter, MD,* Sabine Merkelbach-Bruse, PhD,‡ Hans-Ulrich Schildhaus, MD,‡ Reinhard Buettner, MD, PhD,‡ Edgar Hartung, MD,§ Arnulf Thiede, MD, PhD,* Detlef Meyer, MD, PhD,* Martin Fein, MD, PhD,* Jorn Maroske, MD,‖ and Eva Wardelmann, MD, PhD†*

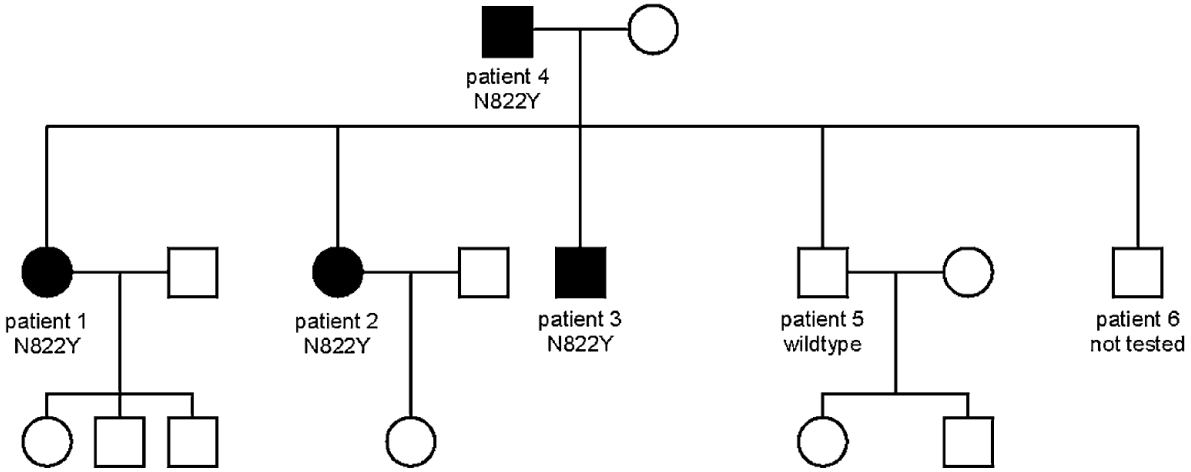


FIGURE 1. Pedigree of the kindred with a mutation in exon 17 of the *KIT* gene. Squares indicate males; circles, females; solid symbols, family members with verified gastrointestinal stromal tumor.

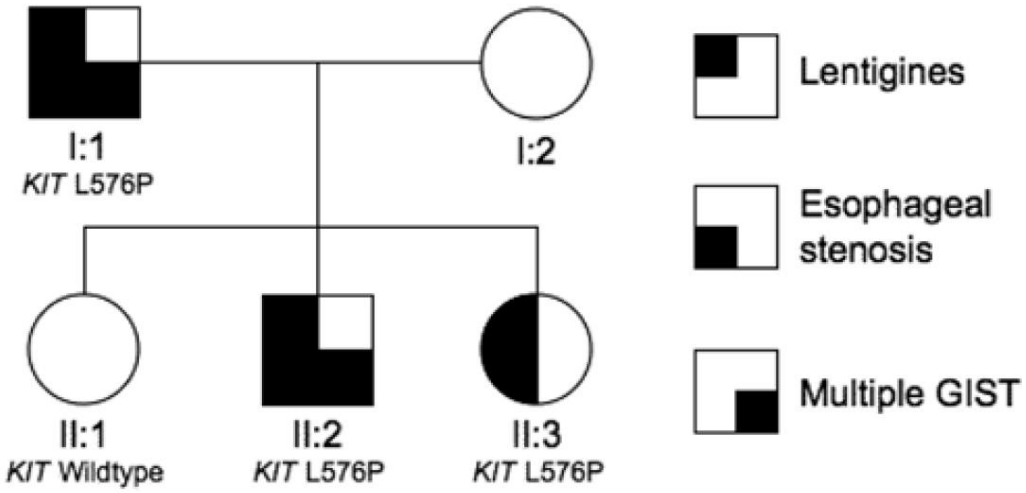
Am J Surg Pathol • Volume 32, Number 10, October 2008

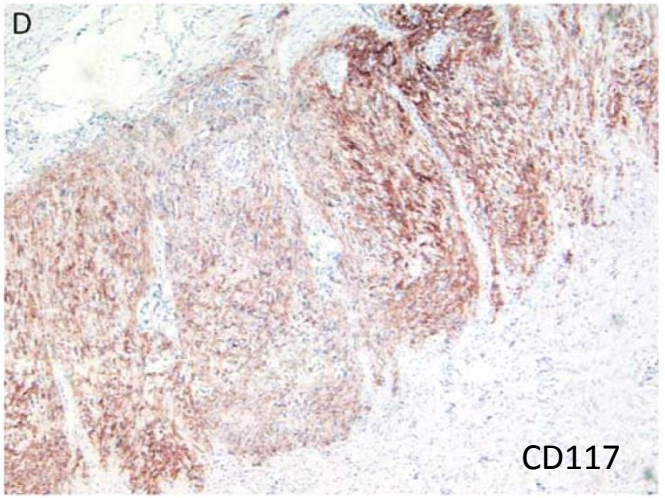
KIT Exon 11

A Novel Germline *KIT* Mutation (p.L576P) in a Family Presenting With Juvenile Onset of Multiple Gastrointestinal Stromal Tumors, Skin Hyperpigmentations, and Esophageal Stenosis

Teresa M. Neuhann, MD,† Veit Mansmann, MD,‡§ Sabine Merkelbach-Bruse, PhD,‖ Barbara Klink, MD,* Achim Hellinger, MD,¶ Heinz-Gert Höffkes, MD,‡ Eva Wardelmann, MD,‖ Hans-Ulrich Schildhaus, MD,‖ and Sigrid Tinschert, MD*#*

Am J Surg Pathol 2013





ICC hyperplasia

KIT Exon 8

Novel Germline Mutation of *KIT* Associated With Familial Gastrointestinal Stromal Tumors and Mastocytosis

KARIN HARTMANN,* EVA WARDELMANN,† YONGSHENG MA,§ SABINE MERKELBACH–BRUSE,† LIANE M. PREUSSNER,* CARLA WOOLERY,§ STEPHAN E. BALDUS,‖ THOMAS HEINICKE,¶ JUERGEN THIELE,‖ REINHARD BUETTNER,† and B. JACK LONGLEY§

GASTROENTEROLOGY 2005;129:1042–1046

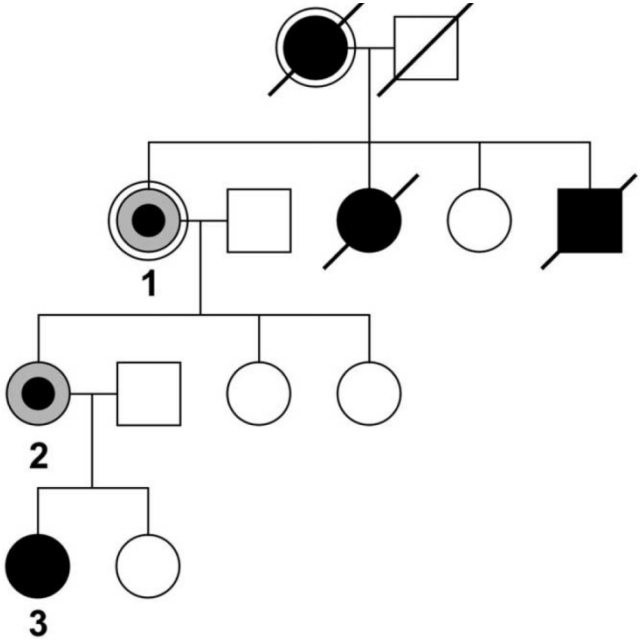


Figure 1. Pedigree of a kindred with familial GISTs, mastocytosis, and dysphagia. Case 1 was diagnosed with GISTs, mastocytosis, and dysphagia; case 2 with GISTs and mastocytosis; and case 3 with mastocytosis only. In addition, 2 deceased siblings of case 1 had mastocytosis and the deceased mother of case 1 had mastocytosis and dysphagia. *Gray symbols*, family members with GISTs; *black symbols*, cases with additional mastocytosis or mastocytosis only; *double outer circles* (case 1 and the mother of case 1), cases with additional dysphagia; *squares*, males; *circles*, females; *symbols with a dash*, dead cases.

Germline *KIT* mutations are associated with GIST, mastocytosis, dysphagia, and hyperpigmentation, but not with germ cells tumors or AML.

A subset of gastrointestinal stromal tumors previously regarded as wild-type tumors carries somatic activating mutations in *KIT* exon 8 (p.D419del)

Sebastian Huss[1], Helen Künstlinger[1], Eva Wardelmann[1], Michaela A Kleine[1], Elke Binot[1], Sabine Merkelbach-Bruse[1], Thomas Rüdiger[2], Jens Mittler[3], Wolfgang Hartmann[1], Reinhard Büttner[1] and Hans-Ulrich Schildhaus[1]

Table 2 Summary of two gastrointestinal stromal tumors with sporadic *KIT* exon 8 mutations

Case No.	*Age/ gender*	*Localization*	*Size (cm)*	*Morphological subtype*	*Risk classification[a] (mitoses)*	*Mutation*	*Adjuvant Treatment*	*DFS (months)*	*Follow up (months)*
1	53/M	Small bowel	5.4	Biphasic (epithelioid and spindled)	High (14/50 HPF)	***KIT*** exon 8 c.1255_1257delGAC p.D419del heterozygous in primary tumor homozygous in metastasis	None	29	Peritoneal metastases (29) lost to follow up with progressive disease (34)
2	67/F	Small bowel	10.0	Spindled	Moderate (1/50 HPF)	***KIT*** exon 8 c.1255_1257delGAC p.D419del heterozygous	Imatinib (400 mg daily)	24	No evidence of disease (24)

DFS, disease-free survival; HPF, high power field.
[a]According to the NCCN-AFIP criteria.[28]

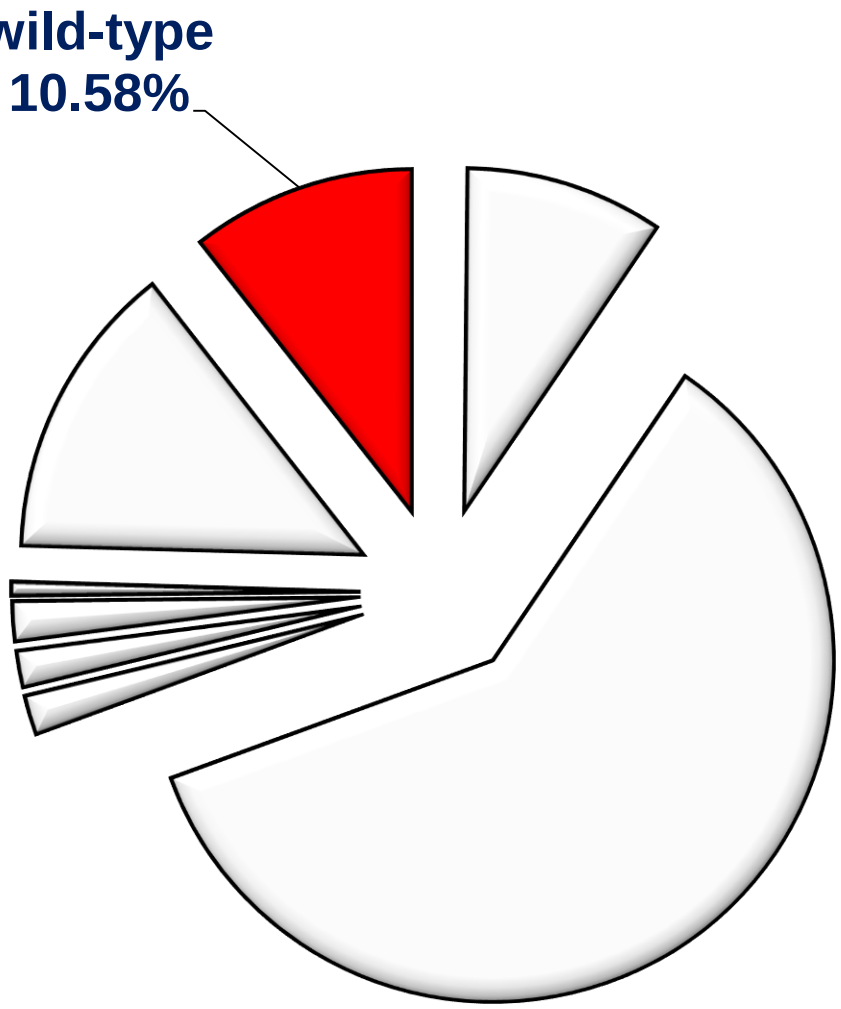
2 cases among 145 wt GIST (1.38% among wt GIST, 0.15% among all)

Ito et al. IJEP 2014: 3 cases among ~ 1000 GIST (0.3%)

Location: small bowel
Biology: can be aggressive

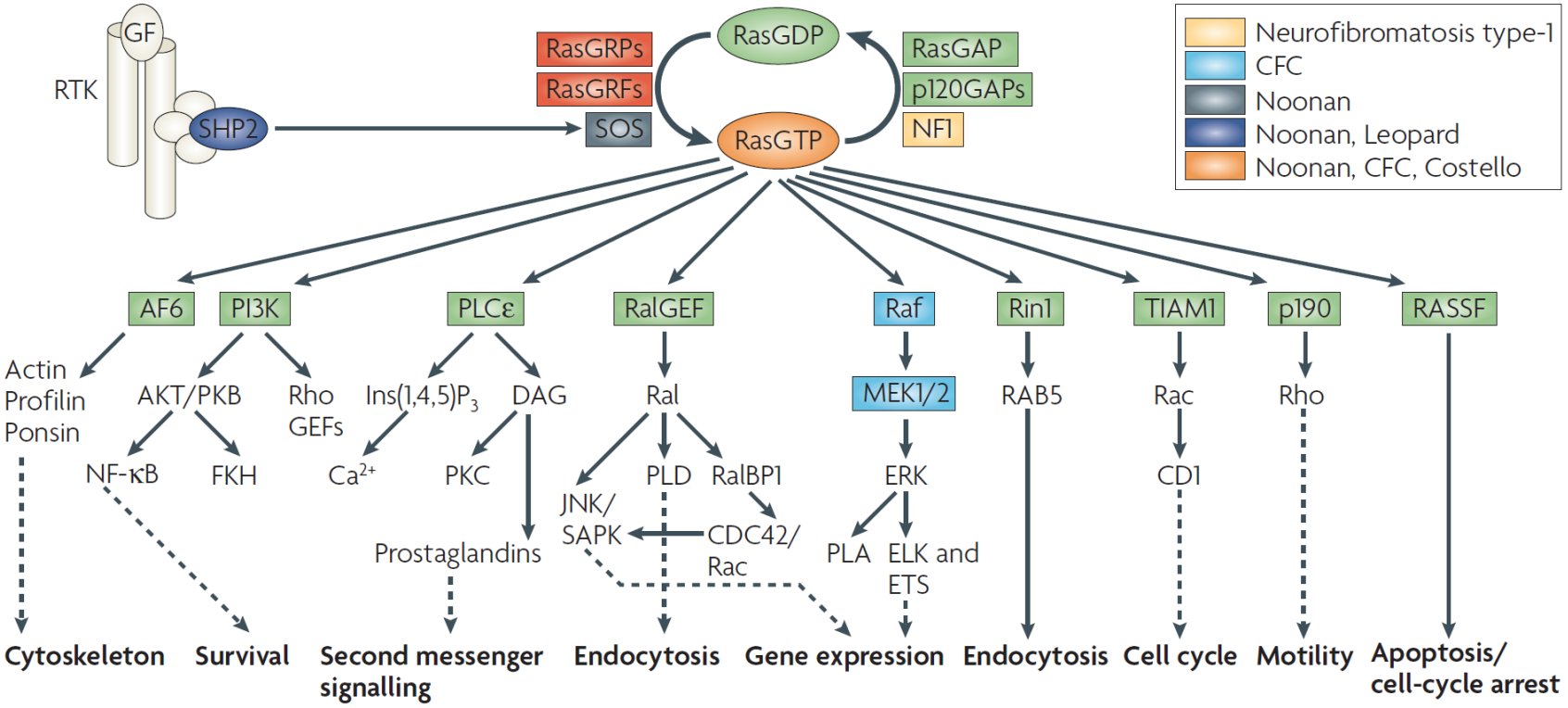
Modern Pathology (2013), 1–9

And beyond *KIT* and *PDGFRA* mutations?



n=1351

Alternative molecular pathways in non *KIT*/non *PDGFRA* mutant GISTs - *NF1* mutations -



Karnoub and Weinberg, Nature reviews 2008

Frequency of GIST in NF1 patients is 7%.

Somatic NF1 loss

Somatic loss of function mutations in *neurofibromin 1* and *MYC associated factor X* genes identified by exome-wide sequencing in a wild-type GIST case

Martin G. Belinsky[1*], Lori Rink[1], Kathy Q. Cai[2], Stephen J. Capuzzi[1,3], Yen Hoang[4,5], Jeremy Chien[5], Andrew K. Godwin[6] and Margaret von Mehren[1]

Table 1 Confirmed somatic mutations

Gene symbol	UniProt accession[a]	Genomic coordinate[b]	Exon	Mutation (cDNA)	Mutation (protein)	Allele frequency	Consensus effect[c]
NF1	P21359	chr17:29665119	44	c.6781_6782insTT	p.His2240Leufs*4	100	n/a[d]
MAX	P61244	chr14:65560437	3	c.160delC	p.Gln54Lysfs*10	91	n/a[d]
RTN4	Q9NQC3	chr2:55200745	8	c.3486_3490delAGAT	p.Asp1163Ilefs2	36	n/a[d]
CCDC66	A2RUB6	chr3:56650054	13	c.1818_1819insCCT	p.Ser606_Lys607insPro	29	n/a[d]
MVD	P53602	chr16:88725087	2	c.112T>A	p.S38T	58	Deleterious
MAFA	Q8NHW3	chr8:144511807	1	c.770A>T	p.Q257L	56	Likely deleterious
RNF123	Q5XPI4	chr3:49751544	31	c.2947T>G	p.Y983D	52	Likely deleterious
SPIN4	Q56A73	chrX:62570610	1	c.89G>T	p.R30L	47	Likely deleterious
SELP	P16109	chr1:169565261	12	c.2003G>T	p.C668F	49	Likely deleterious

[a]http://www.uniprot.org; [b]Hg19; [c]http://www.mypeg.info; [d]Not applicable

BMC Cancer 2015

KRAS mutation in a *KIT* and *PDGFRA* wild type GIST

Novel Oncogene and Tumor Suppressor Mutations in *KIT* and *PDGFRA* Wild Type Gastrointestinal Stromal Tumors Revealed by Next Generation Sequencing

Jaclyn Frances Hechtman,[1]* Ahmet Zehir,[1] Talia Mitchell,[1] Laetitia Borsu,[1] Samuel Singer,[2] William Tap,[3] Alifya Oultache,[1] Marc Ladanyi,[1,4] and Khedoudja Nafa[1]

TABLE 2. Previously Reported Cases of *KRAS*-Mutant GISTs

Case	*KRAS* mutation	*KIT*/*PDGFRA* mutation	Organ	Risk	*KIT* IHC	Response to Imatinib
Miranda et al. (2012)	G12D	*KIT* Δ570-576	Stomach	High	+	Unknown
Miranda et al. (2012)	G12A/ G13D	*KIT* Δ579	Small bowel	Intermediate	+	Unknown
Miranda et al. (2012)	G13D	*PDGFRA* D842V	Stomach	Low	+	Unknown
Antonescu et al. (2013)	G12V	*KIT* Δ557-558	Small bowel	High	−	None
Serrano et al. (2014)	G12R	*KIT* Δ554-559	Stomach	High	+	Limited to *KRAS* wild type nodule
Current	G12V	None	Stomach	High	+	None

Alternative molecular pathways in non *KIT*/non *PDGFRA* mutant GISTs
- BRAF mutations -

Agaram GCC 2008

TABLE 1. Clinical and Pathologic Findings in BRAF mutated GIST patients

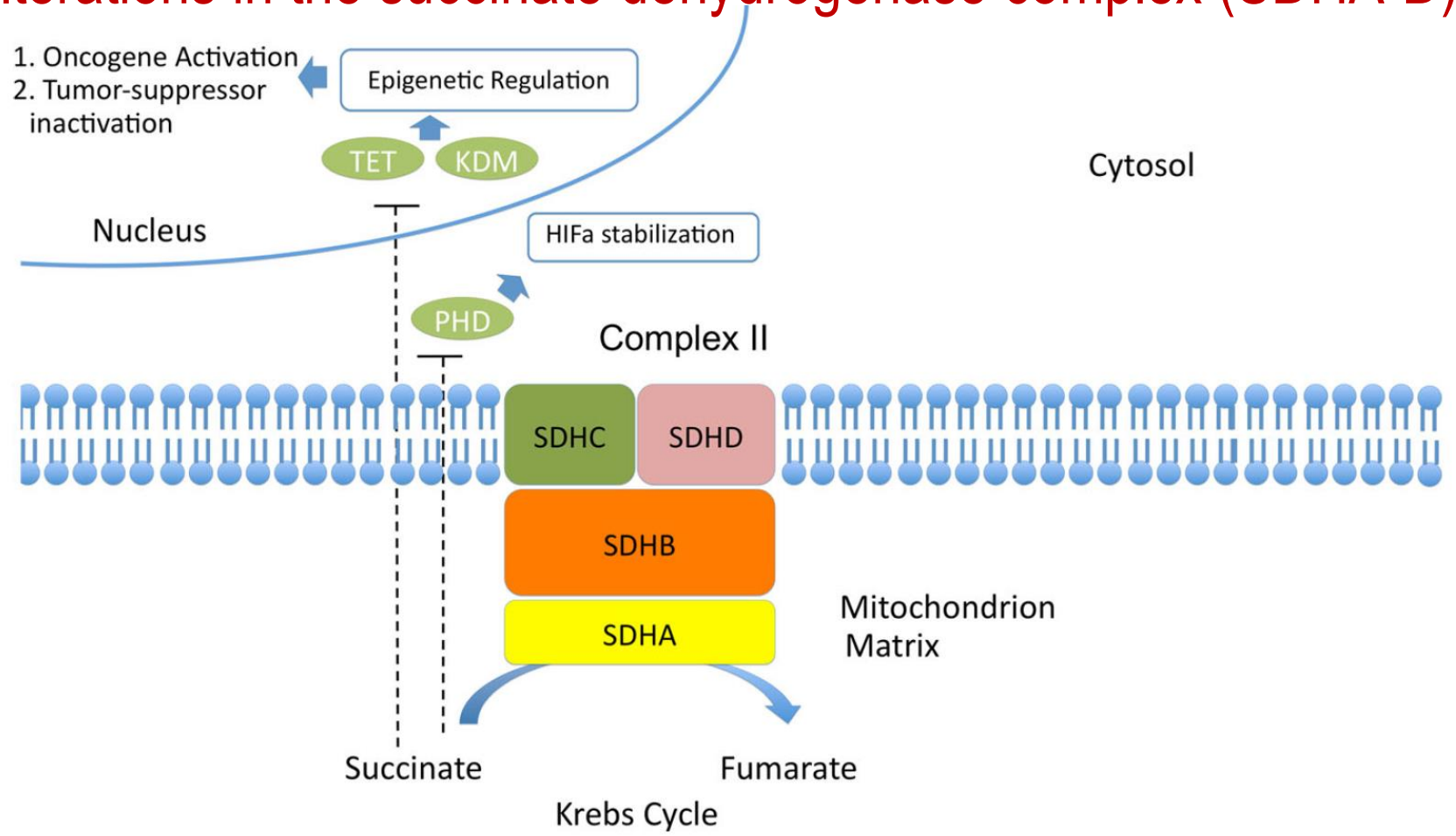
	Age/Sex	Primary Tumor Size (cm)	Primary Tumor Site	MF/50 HPF	Stage at presentation	CD117	PTEN	P16	LFU/mo
1[a]	52/F	10	SB	90	Periton Mets	P	P	N	DOD/18
2	55/F	10	SB	5	Primary	P	NA	NA	NED/9
3	49/F	9	SB	50	Primary	P	P	P	NED/13
4[b]	66/M	20	Stomach	68	Primary	N	N	N	NED/32

F, female; M, male; cm, centimeters; SB, small bowel; periton mets, peritoneal metastases; MF, mitotic figures; HPF, high power fields; P, positive; N, negative; DOD, dead of disease; NED, no evidence of disease; AWD, alive with disease; NA, not available, LFU, last follow-up; mo, months.
[a]Genotyping on normal tissue as well.
[b]Imatinib resistant GIST.

		wt	*KIT-mutant*	*PDGFRA-mutant*
BRAF	wild type	172	228	44
	mutant	7 (3.9%)	0	0
		179	228	44

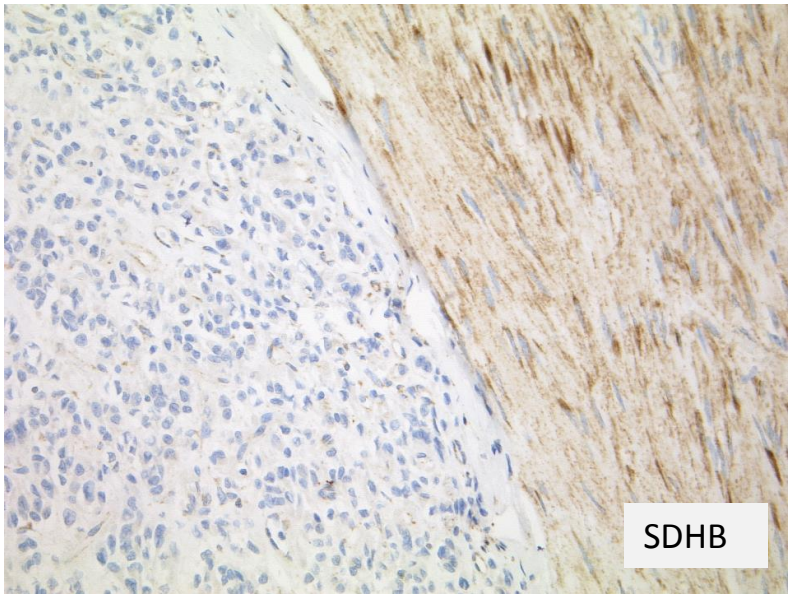
Huss, S et al. (in preparation)

Alternative molecular pathways in non *KIT*/non *PDGFRA* mutant GIST
- alterations in the succinate dehydrogenase complex (SDHA-D) -

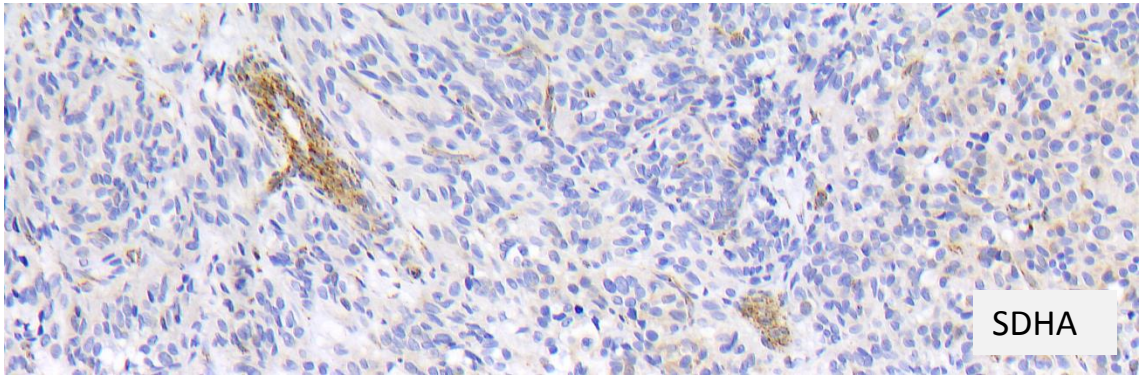


Boikos and Stratakis, Endocrine 2014

SDHB negativity indicates an alteration in the SDH complex

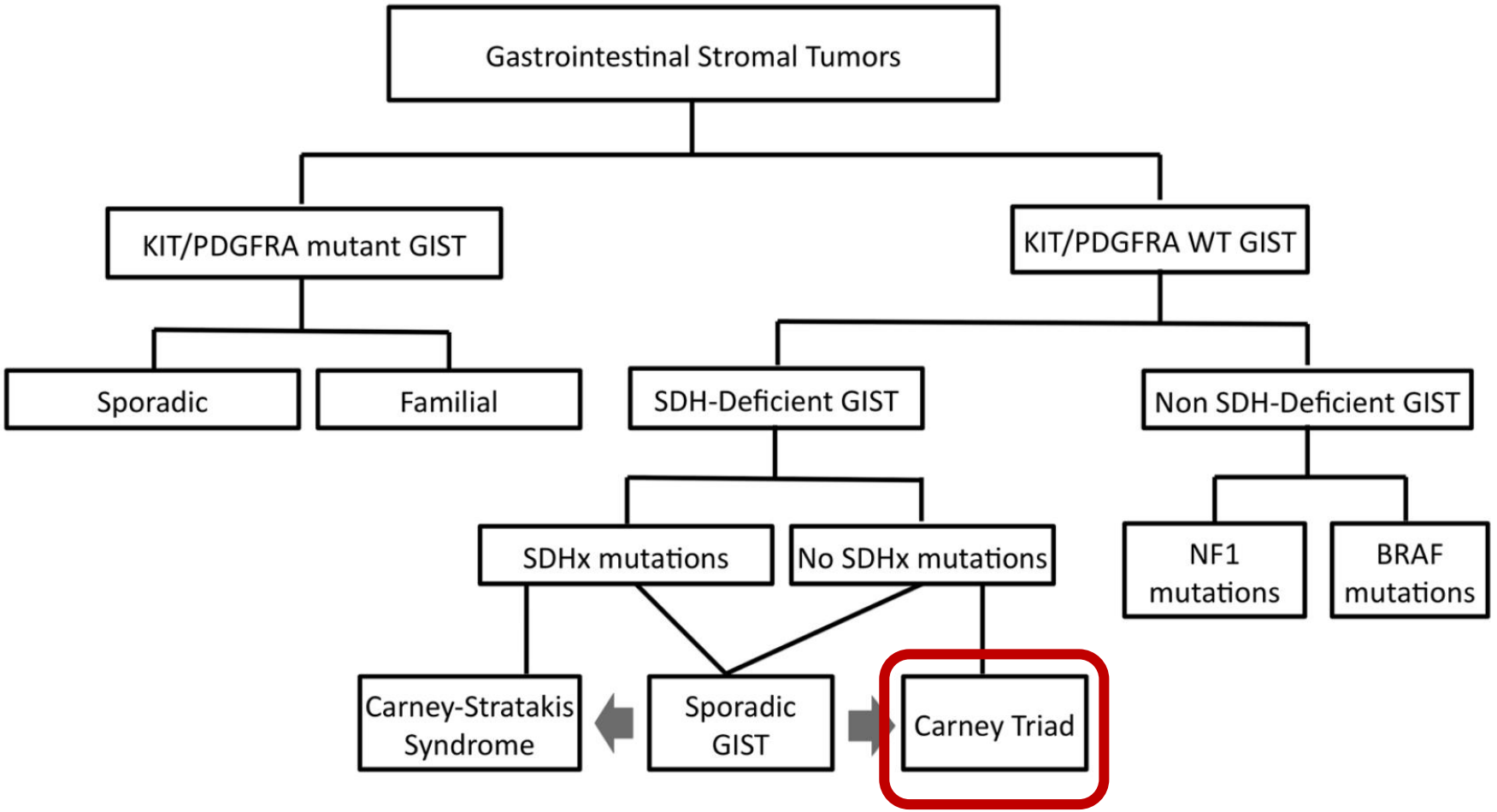


SDHB deficiency indicates an alteration in any of the SDH complex partners (SDHA–D)



SDHA deficiency indicates SDHA alteration

Molecular classification of GIST



Boikos and Stratakis, Endocrine 2014

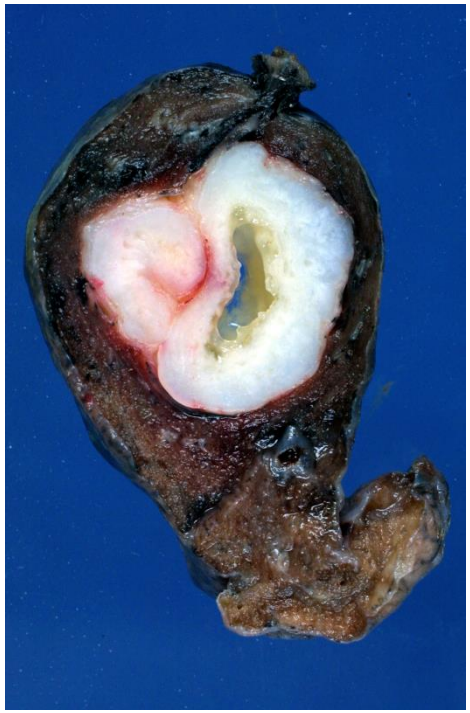
Carney's Triad: GIST + paraganglioma + pulmonary chondroma

- 85% females
- Age at onset: 7-48 (65% before 30)
- Interval between diagnosis of different tumors: up to 26 years
- no mutations in *KIT* or *PDGFRA*

Table 2. **Tumor Combinations Among 79 Patients***

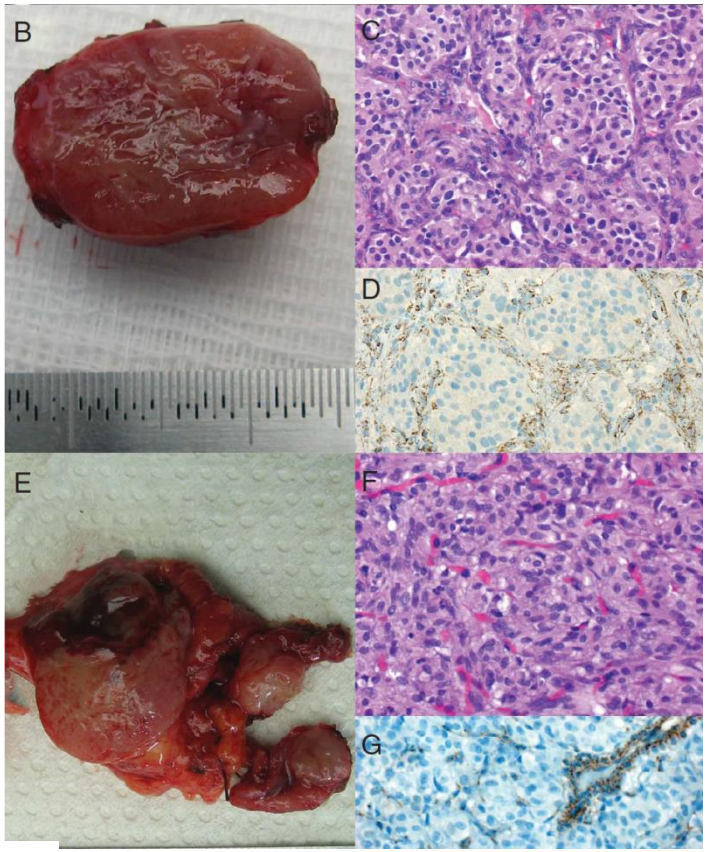
Gastric sarcoma	Pulmonary chondroma	Extra-adrenal paraganglioma	No. (%) of patients
+	+	+	17 (22)
+	+	–	42 (53)
+	–	+	19 (24)
–	+	+	1 (1)

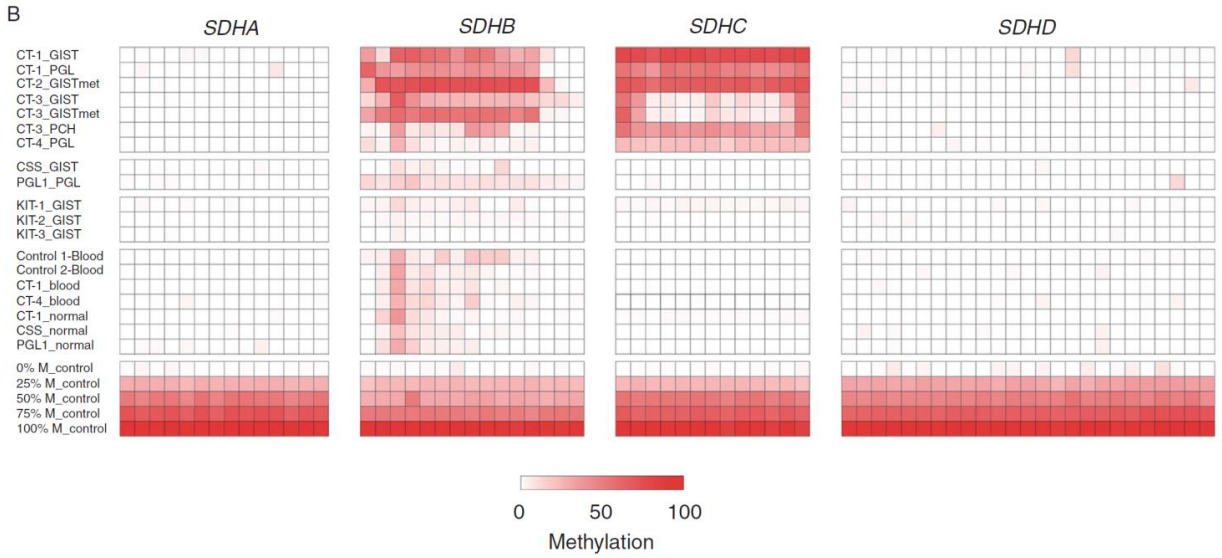
*+ = present; – = absent.



Aberrant DNA hypermethylation of *SDHC*: a novel mechanism of tumor development in Carney triad

Florian Haller, Evgeny A Moskalev, Fabio R Faucz[1], Sarah Barthelmeß, Stefan Wiemann[2], Matthias Bieg[3], Guillaume Assie[4,5], Jerome Bertherat[4,5], Inga-Marie Schaefer[6,†], Claudia Otto[7], Eleanor Rattenberry[8], Eamonn R Maher[8,9], Philipp Ströbel[6], Martin Werner[7], J Aidan Carney[10], Arndt Hartmann, Constantine A Stratakis[1] and Abbas Agaimy





Endocrine-related Cancer 2014

SARCOMA & GIST CONFERENCE 2016

Conclusions

Genes involved in GIST pathogenesis:

KIT exons 8, 9, 11, 13, 17
PDGFRA exons 12, 14, 18
SDHA-D (genetic alterations, epigenetic SDHC dysregulation)
KRAS, BRAF
NF1
….

…other genes to be detected by NGS