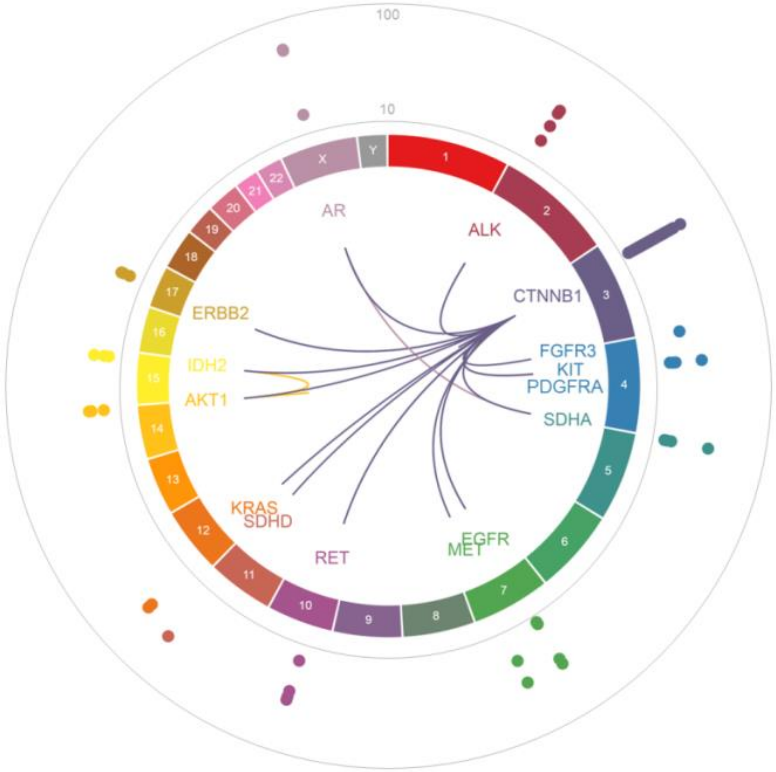
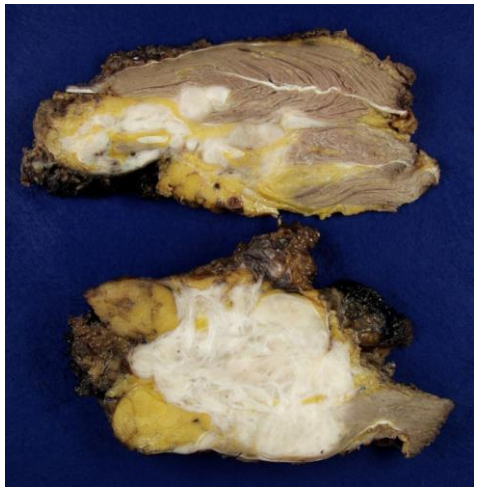




Understanding the biology of desmoids. Are we talking about one disease or different types of desmoids?

2nd february 2020| **Eva Wardelmann, Gerhard-Domagk-Institut für Pathologie, Universitätsklinikum Münster**

DISCLOSURE SLIDE

- I received honoraria and travel grants from Nanobiotix, Milestone/Menarini, New Oncology, Lilly, Novartis Oncology, Roche and Bayer

- I serve as vice president of the International Academy for Pathology (IAP), as member of the management board of the German Society of Pathology (DGP), as member of the steering committee of the German S3-Guidelines for Sarcomas (under the auspices of the German Cancer Society - DKG), as founder member of the German Sarcoma Foundation (DSS) and as chair of the Subcommittee for Pathology and Translational Research of the STBSG (EORTC)

What is a desmoid/fibromatosis?

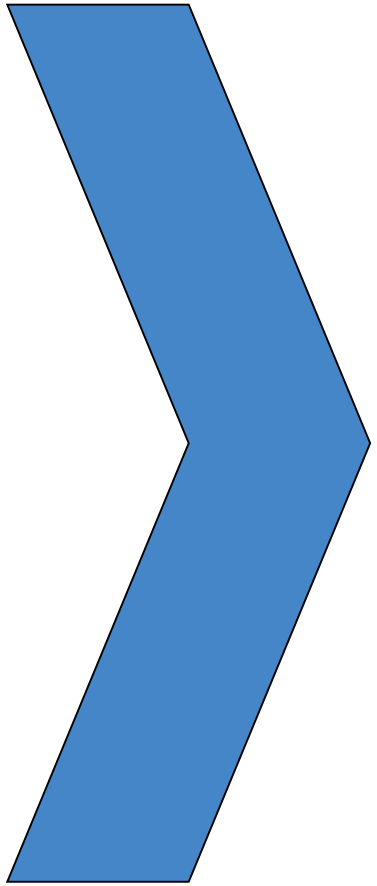
First, let‘s separate both terms:

desmoid:

- ✓desmos (greek) band, chain, bond
- ✓-oid: comparable, similar

fibromatosis:

- ✓ fibra: (latin) fiber
- ✓ -osis: chronic disease



desmoid fibromatosis

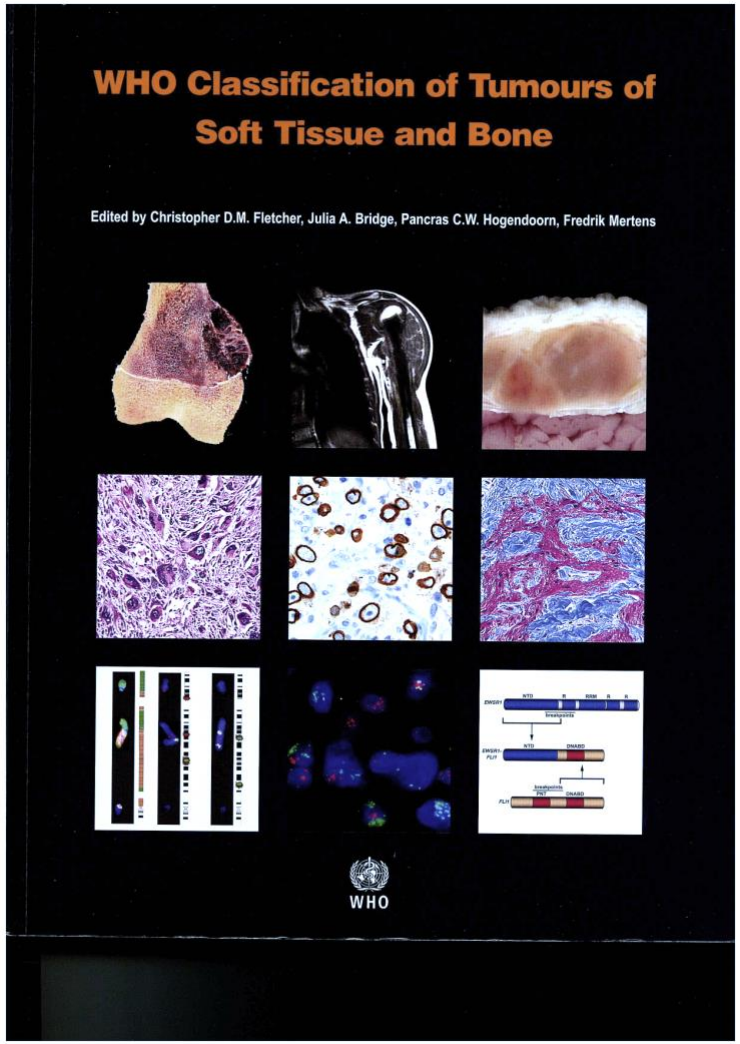
desmoid-type fibromatosis

fibromatosis

desmoid

desmoid tumor

Let‘s look into the WHO classification



2013

Benign
- Nodular fasciitis
- Proliferative fasciitis
- Proliferative myositis
- Myositis ossificans
- Fibro-osseous pseudotumour of digits
- Ischemic fasciitis
- Elastofibroma
- Fibrous hamartoma of infancy
- Fibromatosis colli
- Juvenile hyaline fibromatosis
- Inclusion body fibromatosis
- Fibroma of tendon sheath
- Desmoplastic fibroblastoma
- Mammary-type myofibroblastoma
- Calcifying aponeurotic fibroma
- Angiomyofibroblastoma
- Cellular angiofibroma
- Nuchal-type fibroma
- Gardner fibroma
- Calcifying fibrous tumour

Intermediate (rarely metastasizing)
- Dermatofibrosarcoma protuberans
- Fibrosarcomatous dermatofibrosarcoma protuberans
- Pigmented dermatofibrosarcoma protuberans
- Solitary fibrous tumour
- Solitary fibrous tumour, malignant
- Inflammatory myofibroblastic tumour
- Low grade myofibroblastic sarcoma
- Myxoinflammatory fibroblastic sarcoma / Atypical myxoinflammatory fibroblastic tumour
- Infantile fibrosarcoma

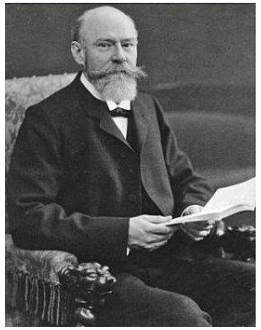
Malignant
- Adult fibrosarcoma
- Myxofibrosarcoma
- Low-grade fibromyxoid sarcoma
- Sclerosing epithelioid fibrosarcoma

Intermediate (locally aggressive)
- Palmar / plantar fibromatosis
- Desmoid-type fibromatosis
- Lipofibromatosis
- Giant cell fibroblastoma

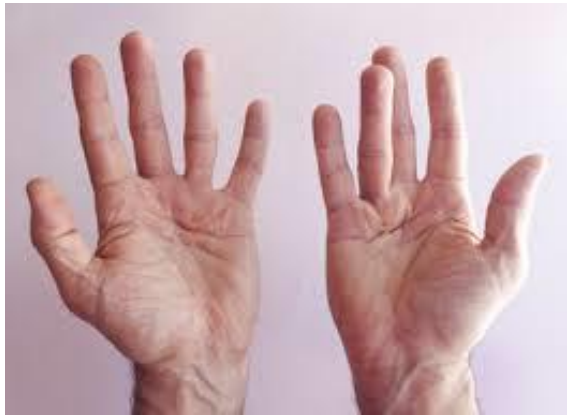
Who get‘s a desmoid?

- 5-6 cases per 1 million per year
- Peak age: 30 to 40 years
- female predominance

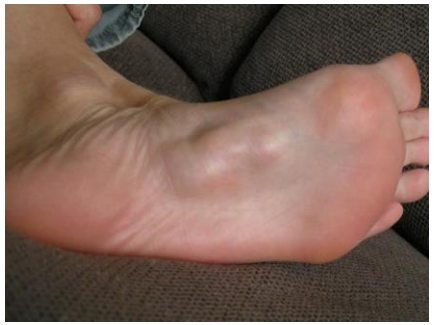
Guillaume Dupuytren 1777-1835
French surgeon



Georg Ledderhose 1855-1925
German surgeon

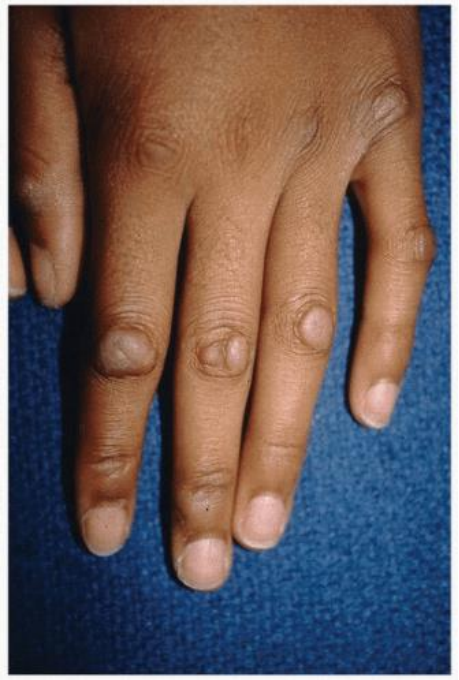


©perfectmatch - stock.adobe.com



https://www.dupuytren-online.de/images/
Ledderhose_Debbie_beforeRT.jpg

Knuckle pads

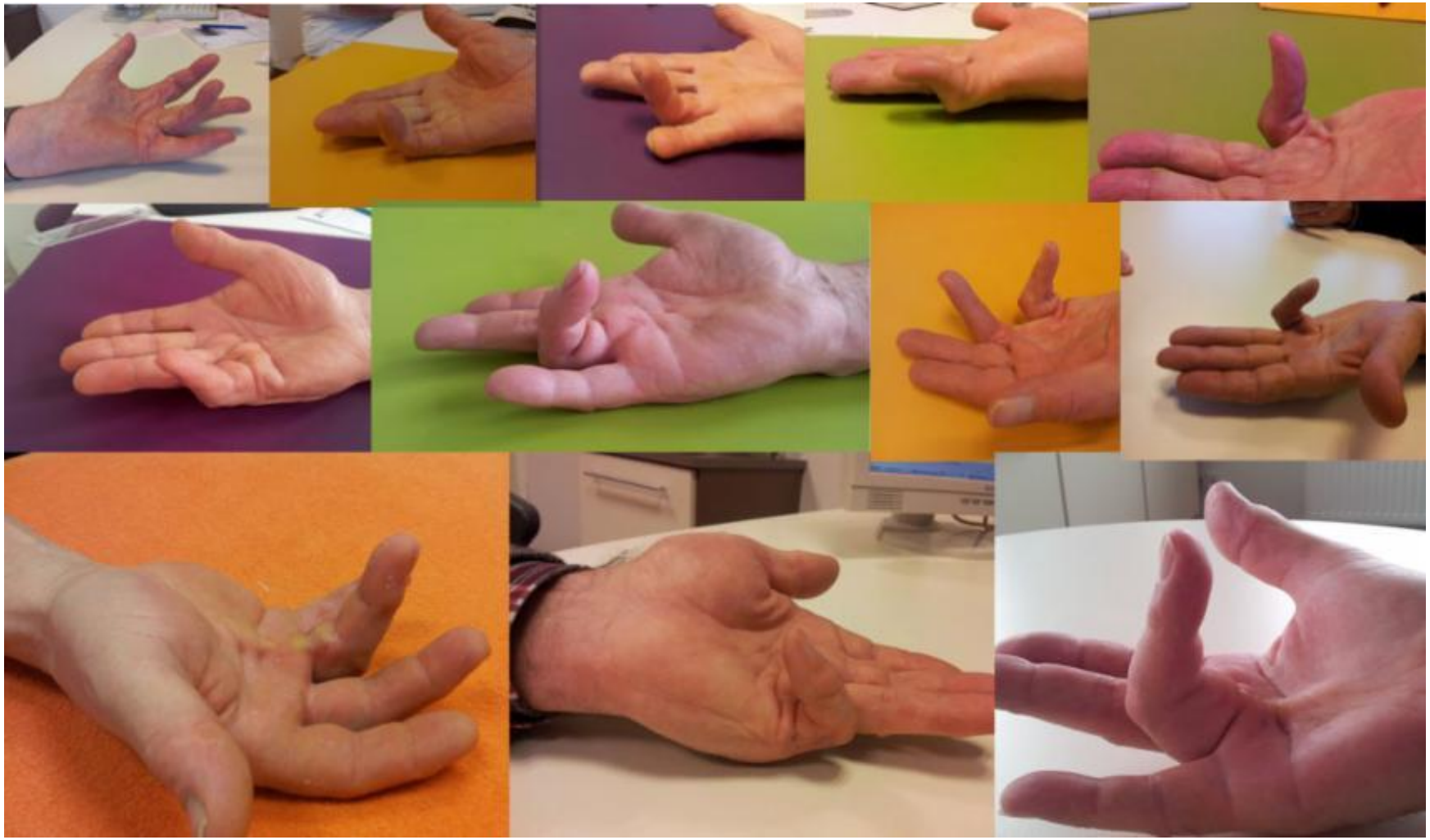


https://i2.wp.com/plasticsurgerykey.com/
wp-content/uploads/2018/10/C3-FF7-2.gif?w=960

What is the difference between superficial (plantar and palmar) fibromatosis and desmoid-type fibromatosis?

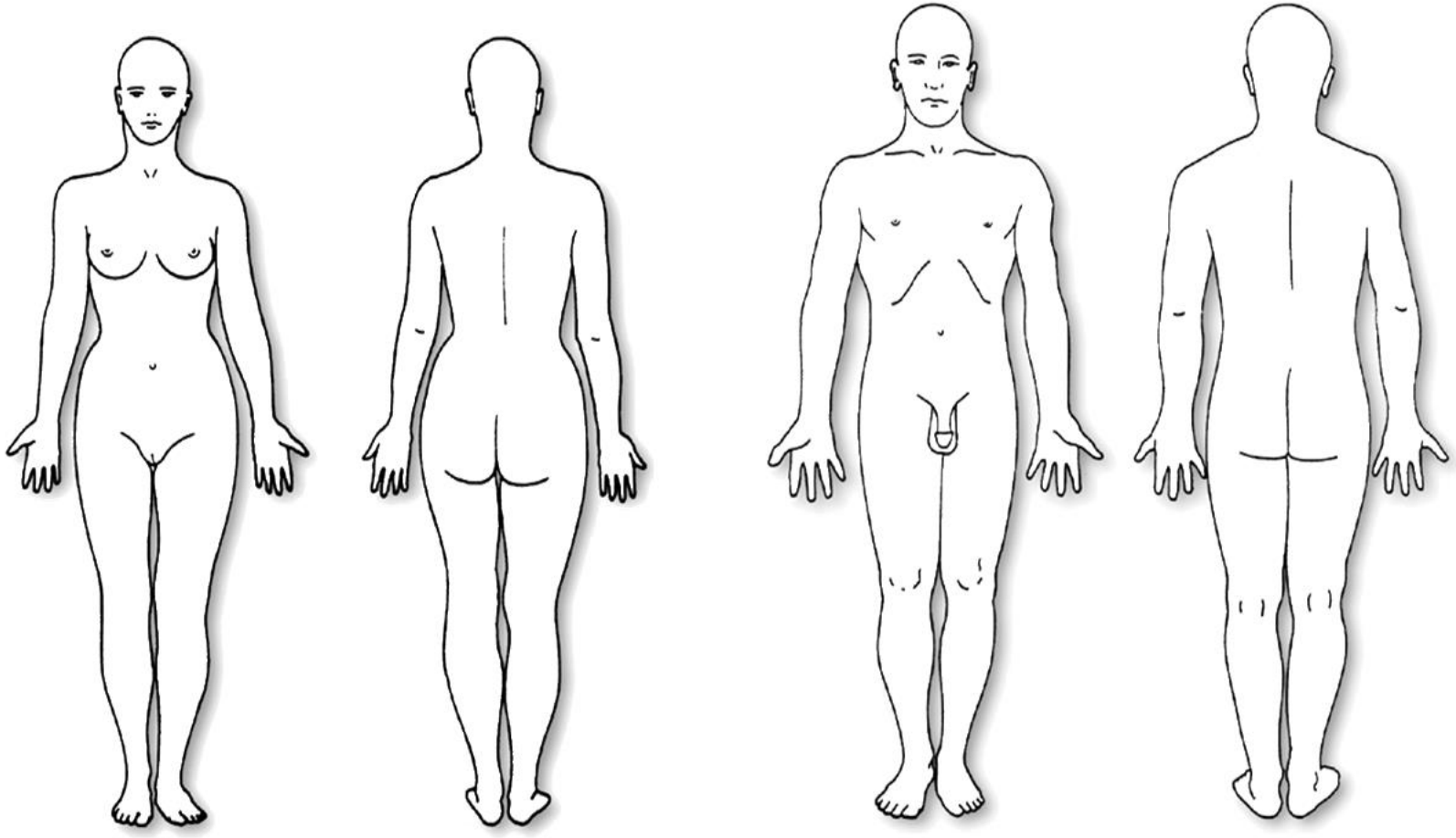
- Superficial fibromatosis occurs on hands and feet (Dupuytren disease and Ledderhose disease)
- Microscopically, superficial fibromatosis looks very similar compared to desmoid-type fibromatosis
- Superficial fibromatosis has some common features with desmoid fibromatosis but no *CTNNB1* mutations
- Incidence increases with age > 30 years
- male predominance

Dupuytren disease



https://www.handchirurgie-hofbeck.de/wp-content/uploads/2017/05/wsb_853x499_dupuytrencollage.jpg

Where do desmoid-type fibromatoses occur?



Everywhere in the body!

What about the biological behavior of desmoid-type fibromatosis?

in the www:

aggressive

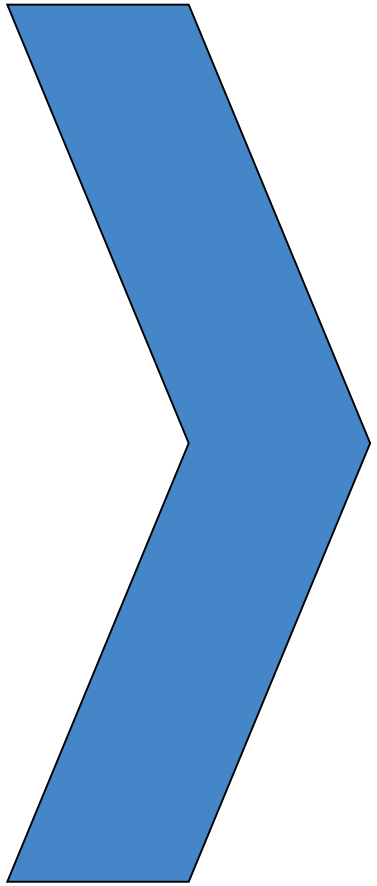
benign

semi-malignant

intermediate

invasive

…



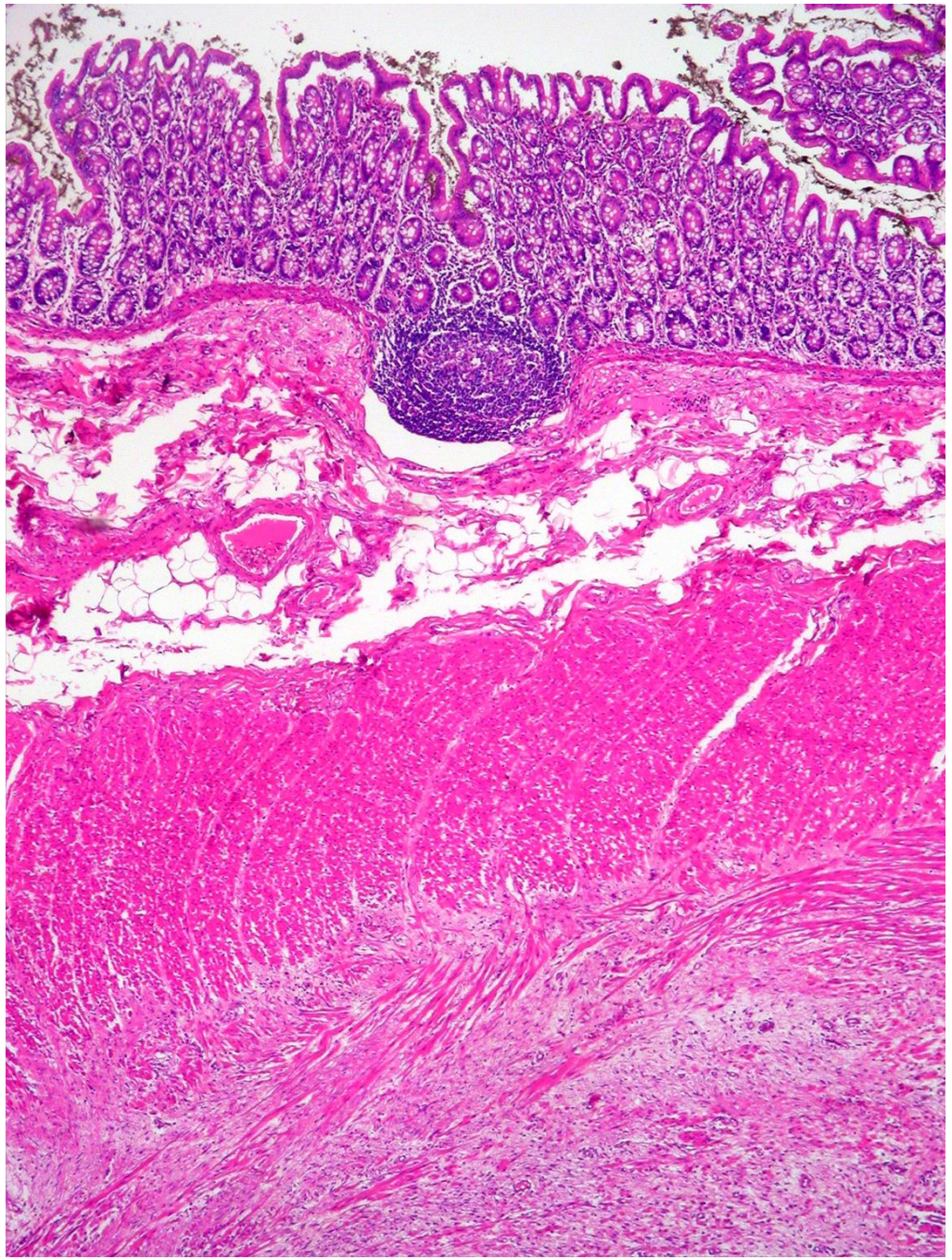
WHO classification:

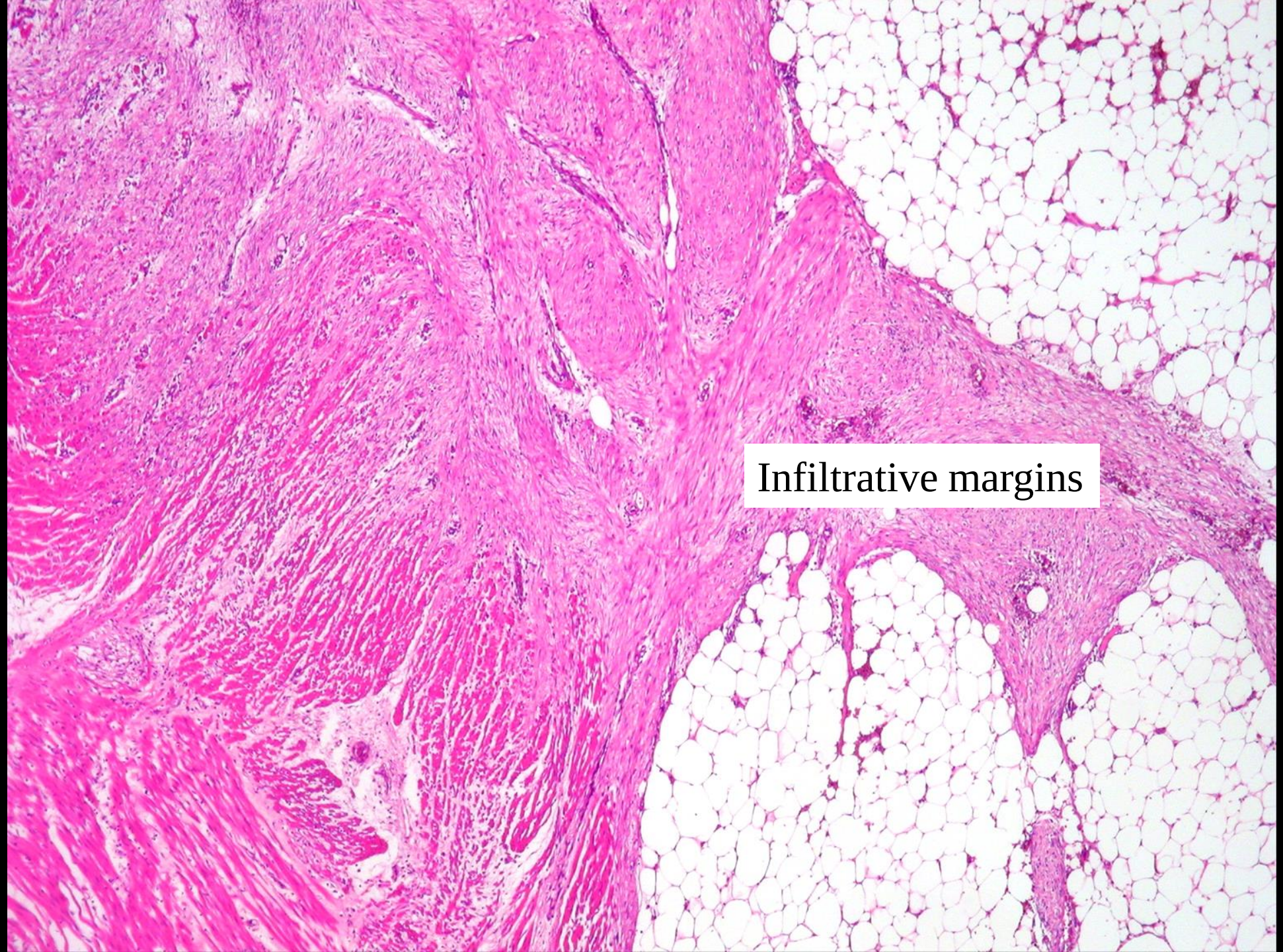
intermediate biology
(locally aggressive)

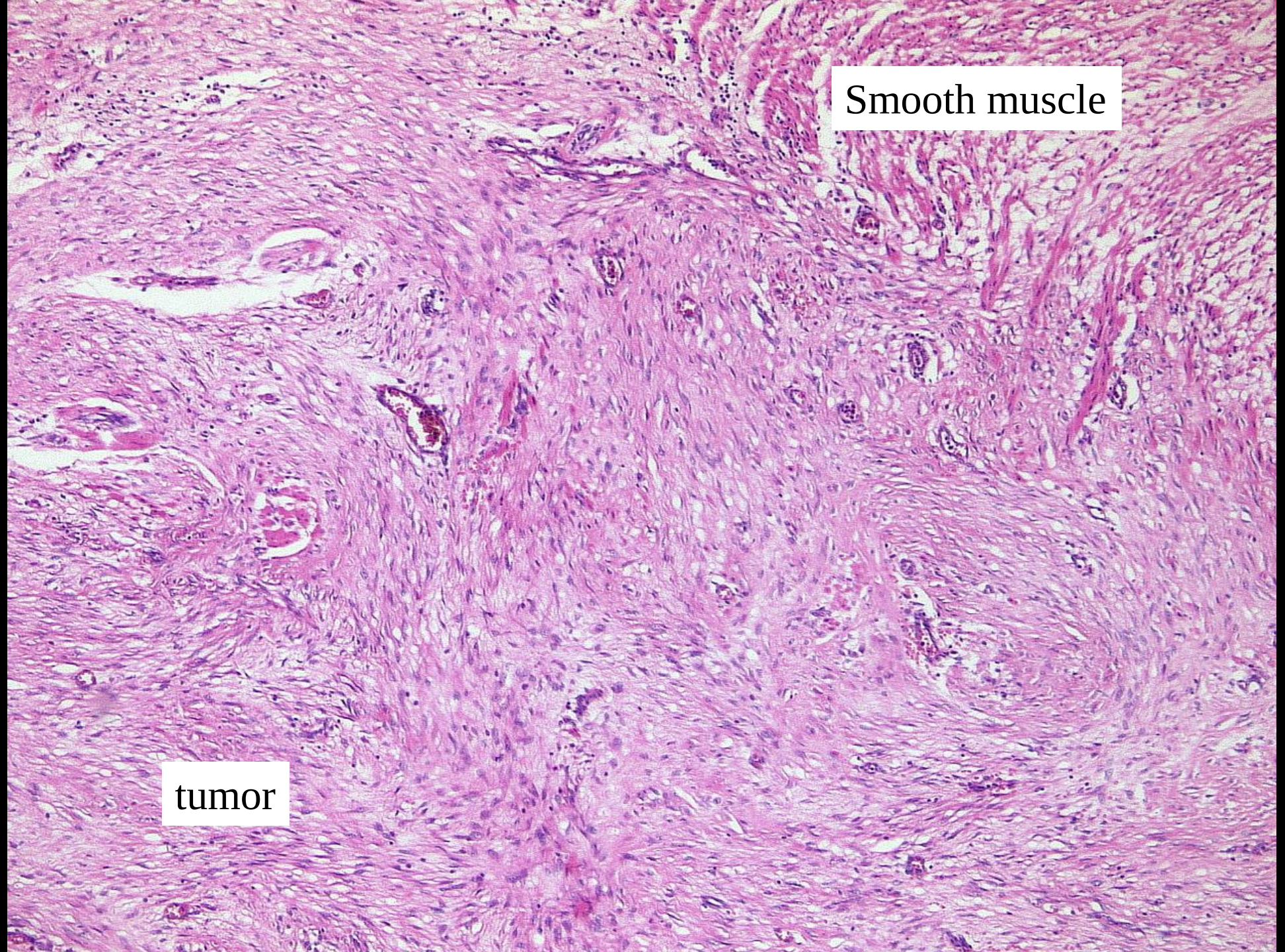
- can regress
- can recur
- do not metastasize
- can affect quality of life

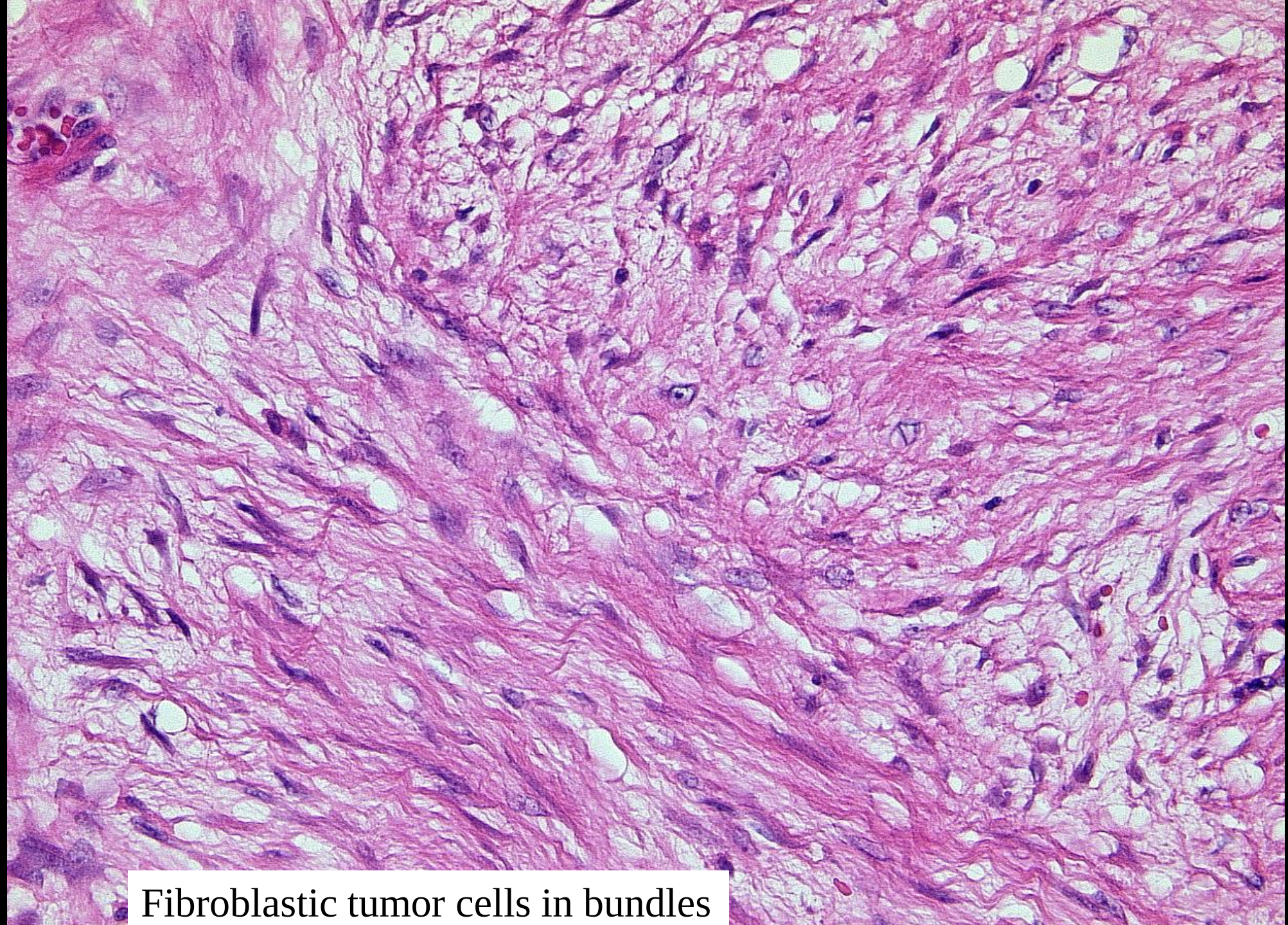
UKM
Universitätsklinikum
Münster

39-years old female patient with a 6 cm measuring nodular tumor of the transversal colon connected to the abdominal wall

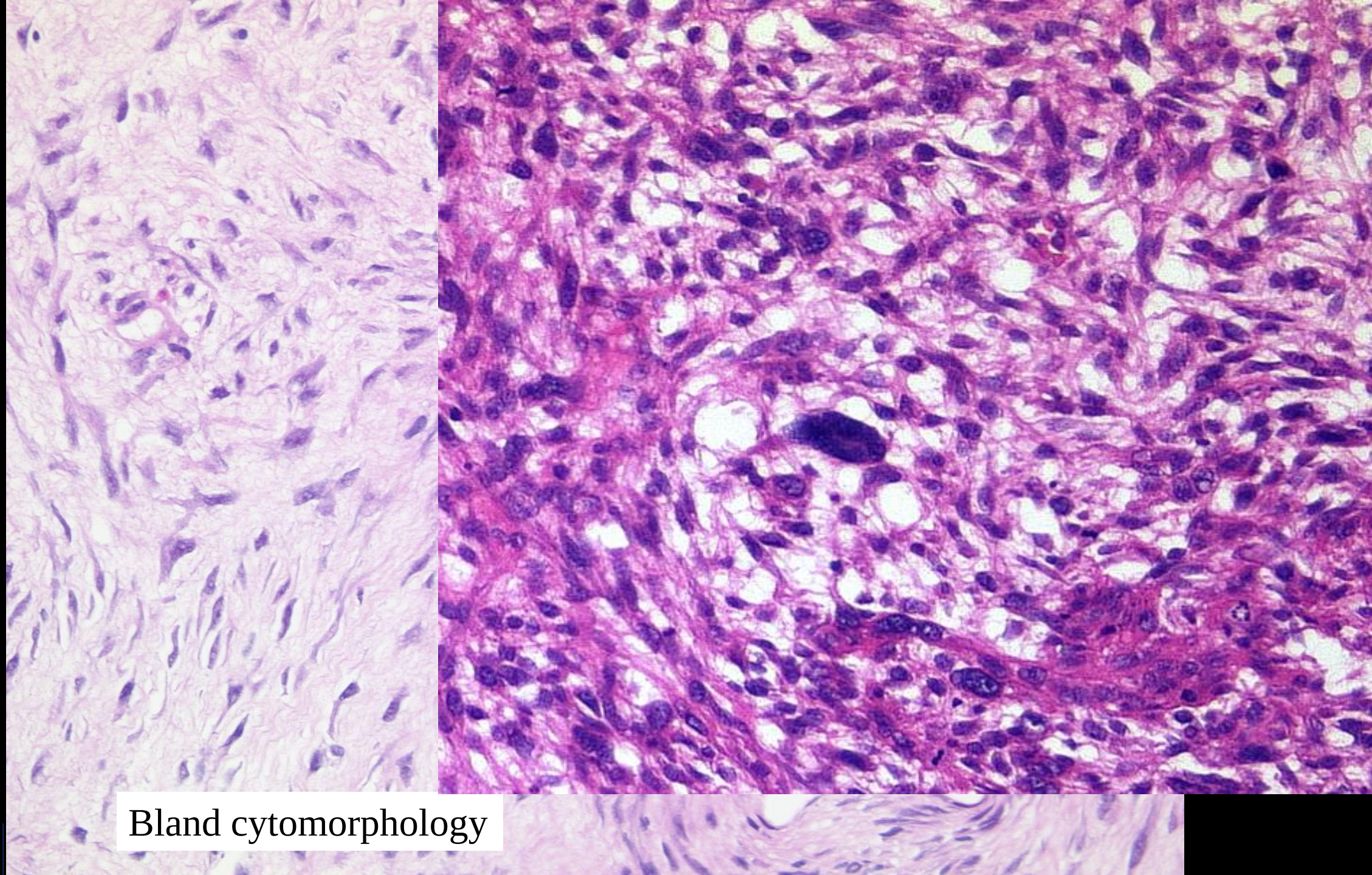



Infiltrative margins

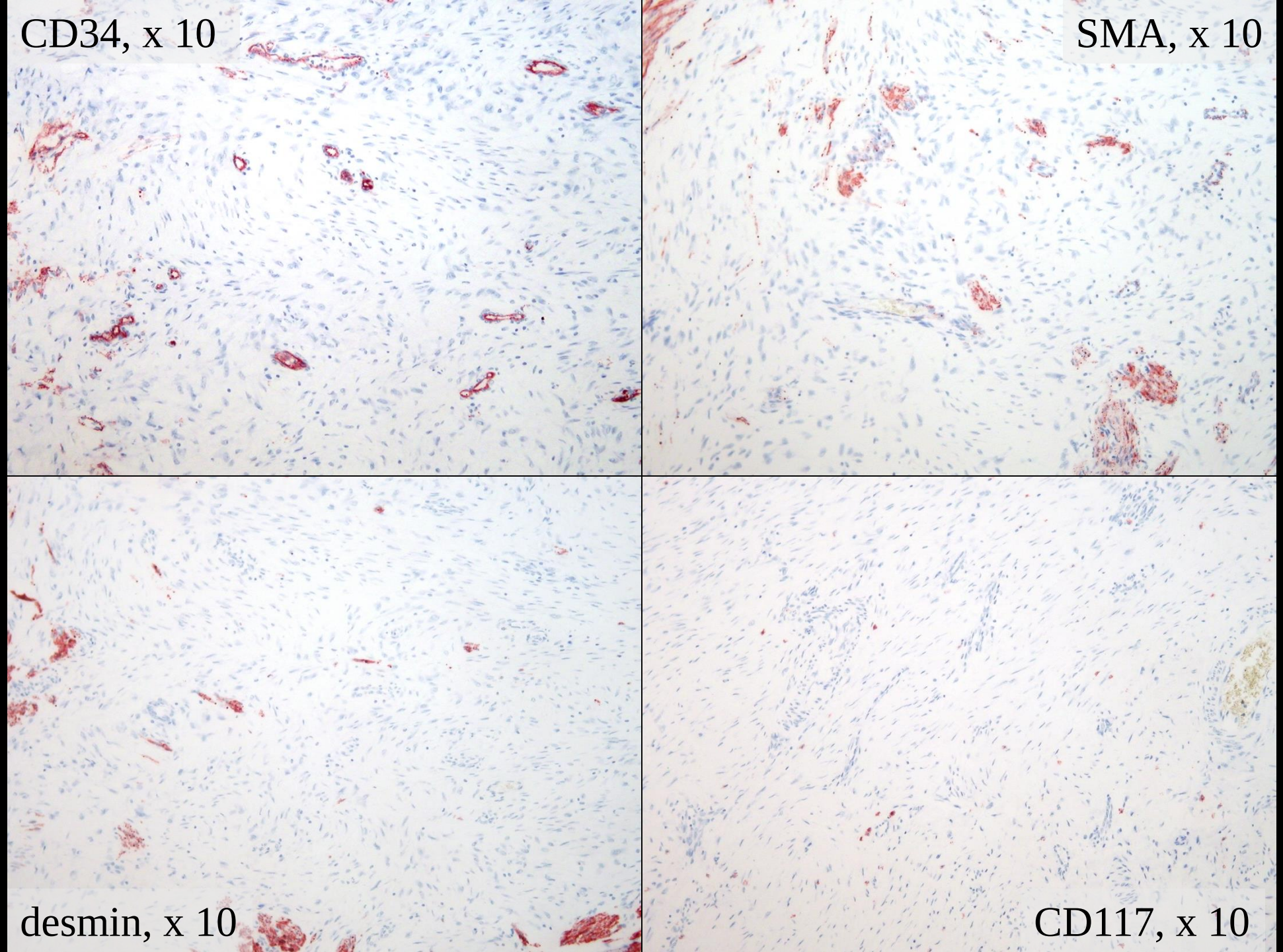

Smooth muscle
tumor

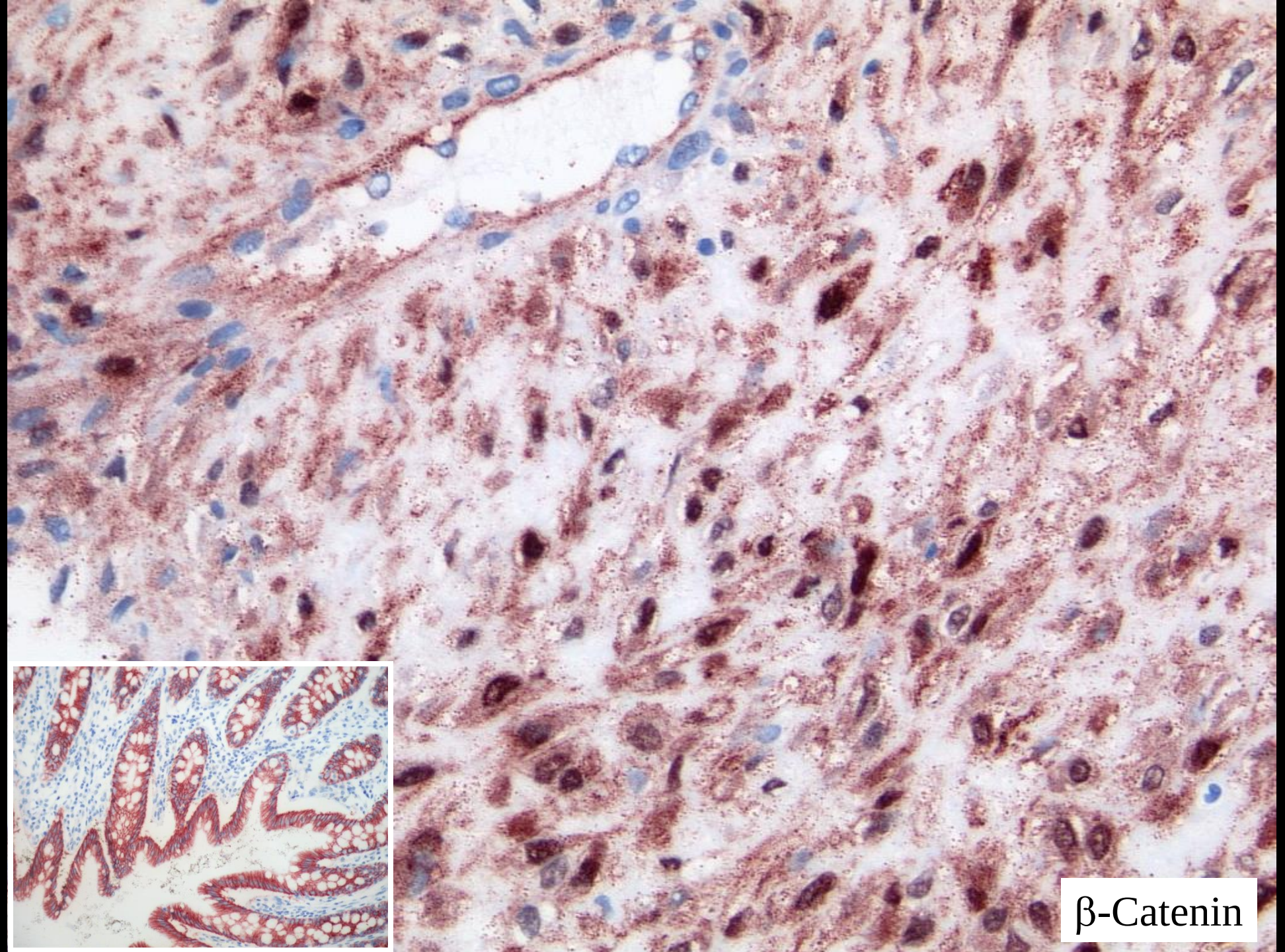


Fibroblastic tumor cells in bundles



Bland cytomorphology


CD34, x 10
SMA, x 10
desmin, x 10
CD117, x 10



β-Catenin

Important differential diagnoses

Dedifferentiated liposarcoma

MDM2/CDK4 overexpression/amplification

GIST (spindle cell type)

KIT+; CD34+; DOG-1+;

Typically connected to the lamina muscularis propria of the tubular GI tract, often protrudes into the mesentery

Retroperitoneal fibrosis (Ormond's disease)

hyalinised collagen

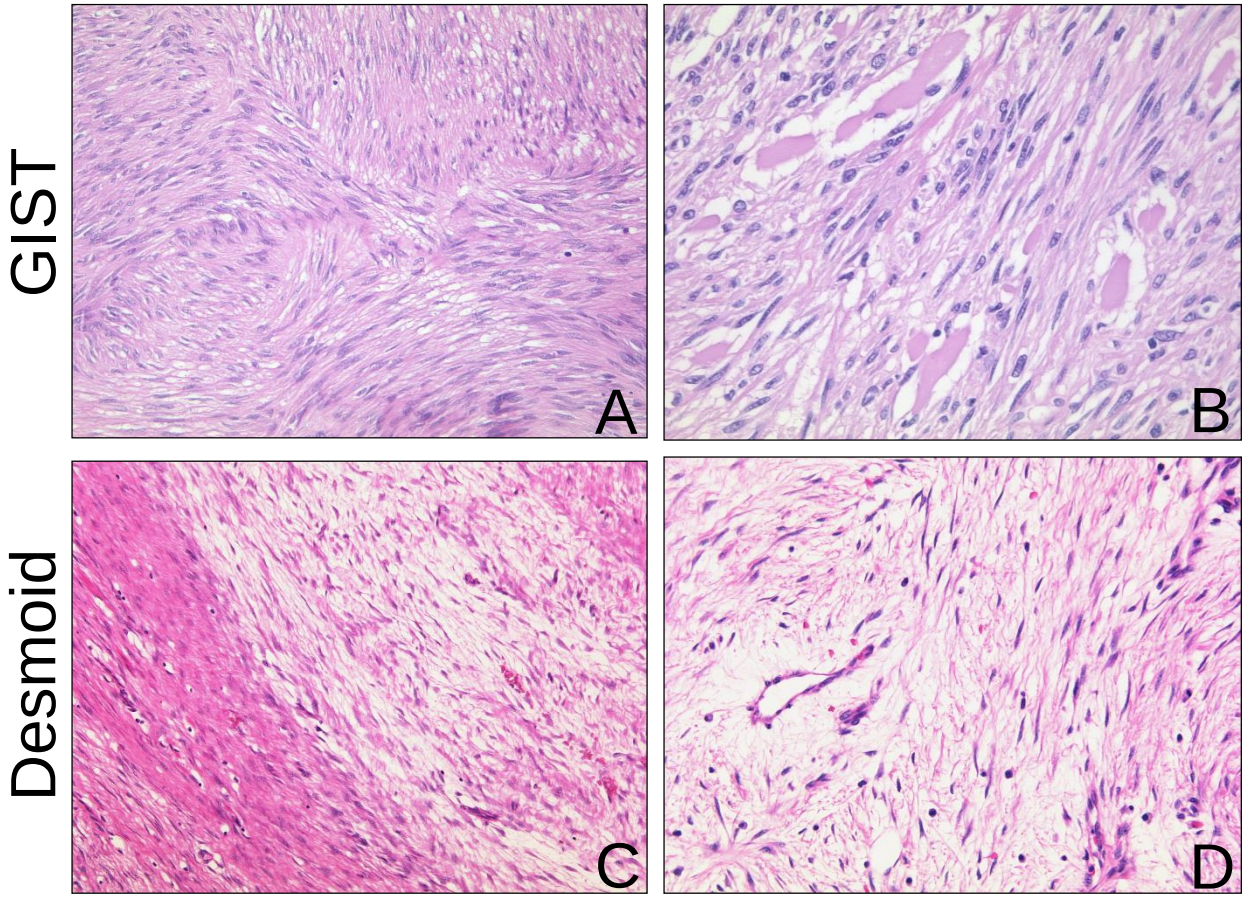
Lymphoplasmocytic infiltrate

no nuclear ß-catenin expression

IgG4-expressing plasma cells increased

Huss, S. et al. Histopathology 2013

Morphologic similarities of GIST and fibromatosis



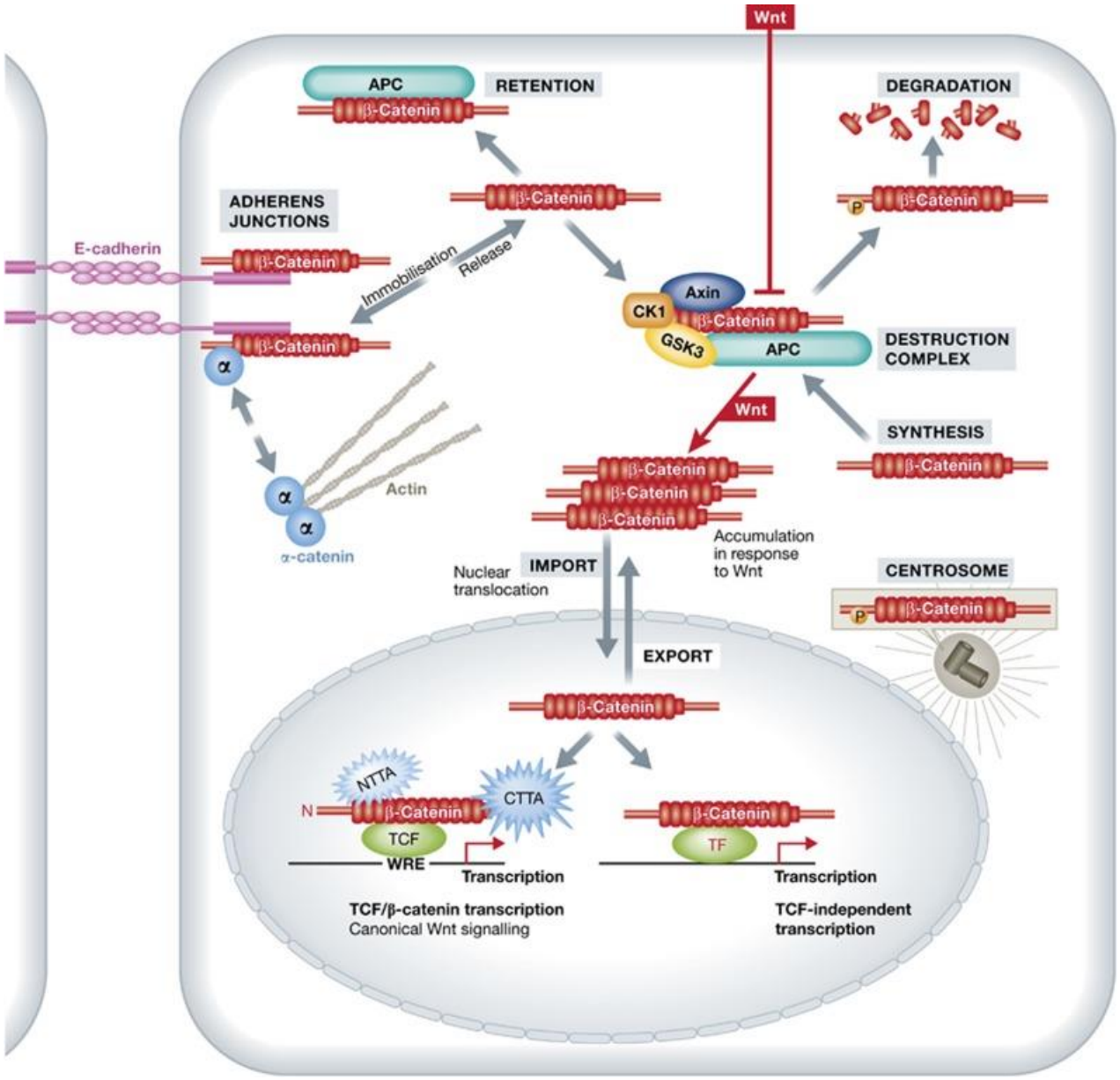
Differential diagnosis can be made easily by immunohistochemistry:

- ß-catenin
- CD34
- DOG 1
- CD117

The life of ß-catenin in the cell

Functions:

- dual role in development – signalling and structural
- establishment of body axis
- tissue homeostasis
- cell renewal
- regeneration
- …

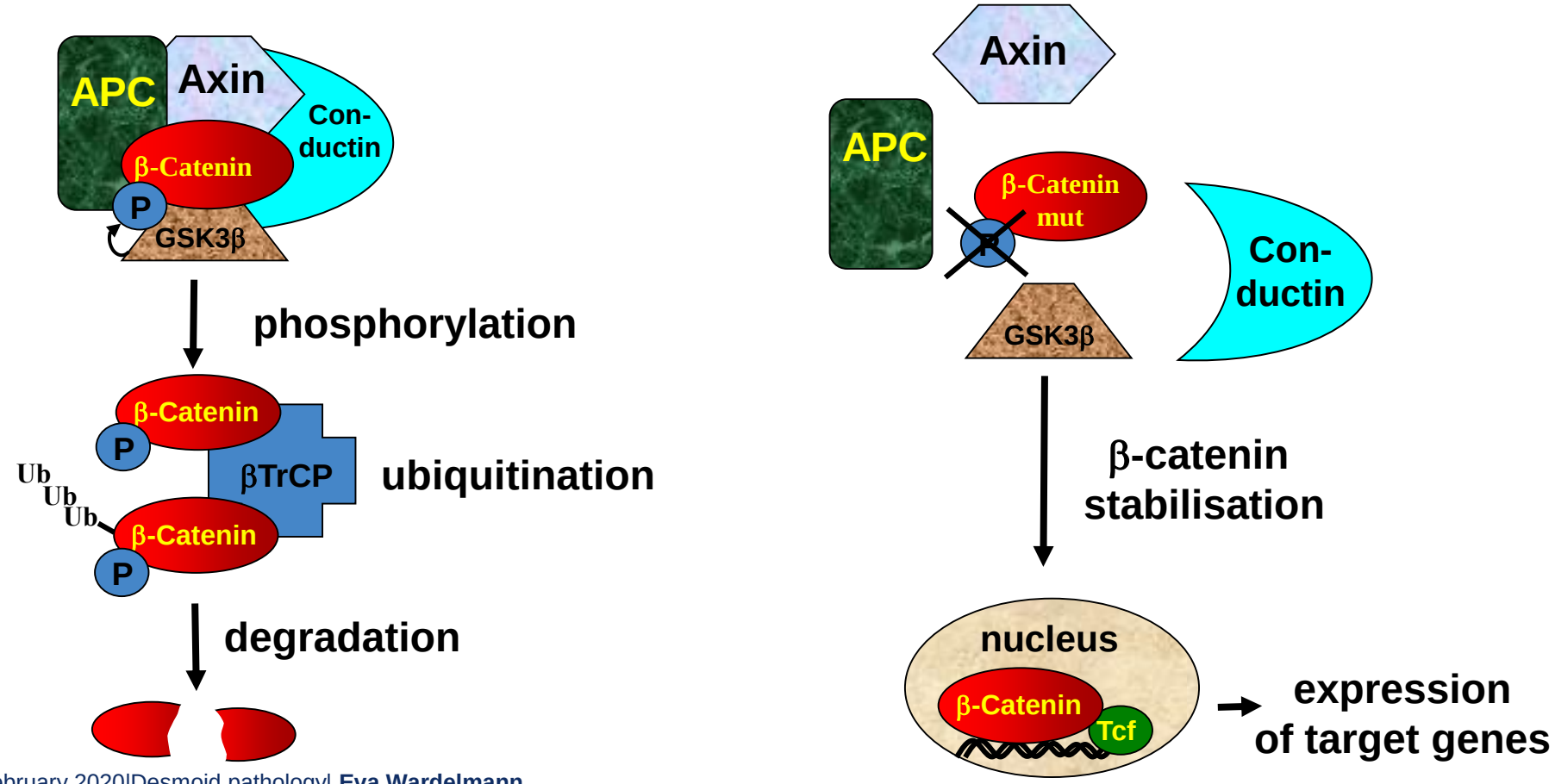


Valenta et al. EMBO J 2012

ß-catenin level and location in the cell varies…

Physiologic degradation

Mutation associated inhibition of degradation



Courtesy of Arend Koch

How/where ß-catenin is regulated

B

MODIFICATION	SITES	ENZYME	FUNCTION (enhancement of)	REFERENCES
Ser/Thr phosphorylation	S33, S37	GSK3	Degradation, provides sites for β-TrCP	Winston *et al* (1999) Liu *et al* (1999)
	T41	GSK3	Degradation, phosporylation relay site	Wu and He (2006)
	S45	CK1	Degradation, priming for GSK3	Liu *et al* (2002)
	T112	CK2	Adhesion, promotes α-catenin binding	Bek and Kemler (2002)
	T120	PKD1	Inhibition of signalling by immobilization of β-catenin in trans-Golgi	Du *et al* (2009) Du *et al* (2012)
	S191	JNK2	Nuclear translocation	Wu *et al* (2008)
	S246	Cdk5	Inhibition of APC binding (via Pin 1)	Muñoz *et al* (2007)
	T393	CK2	Signalling, promotes stability	Song *et al* (2003)
	S552	Akt, PKA	Signalling	Fang *et al* (2007)
	S605	JNK2	Nuclear translocation	Wu *et al* (2008)
	S675	PKA	Signalling, enhancement of CBP binding	Fang *et al* (2007) van Veelen *et al* (2011)
	S675	PAK (p21 activ. kinase)	Signalling, promoting stability and transcription	Zhu *et al* (2012)
	S23, S29	CK2 (?)	Stability (?)	van Noort *et al* (2002)
	(?) (in *Drosophila*)	Hipk, HipK2	Promoting stability of Armadillo (opposite in other system reported)	Lee *et al* (2009) Kim *et al* (2010)
	S764, S802, S827 (sites not in vertebrates)	NLK	Connecting Armadillo–E-cadherin complex with Wnt/PCP pathway	Mirkovic *et al* (2011)
Tyr phosphorylation	Y654	Src	Signalling, reduces cadherin binding allows TBP binding	Huber and Weis, (2001) van Veelen *et al* (2011)
	Y142	Fer/Fyn; Met	Signalling, reduces α-catenin binding	Brembeck *et al* (2004) Bustos *et al* (2006)
	Y86, Y654	Bcr-Abl, Abl	Signalling, stabilizing β-catenin	Coluccia *et al* (2007)
	Y333	Src (EGFR mediated)	Signalling, promotes nuclear function in response to EGF (Wnt independent)	Yang *et al* (2011 b)
	Y489	Abl	Signalling, disrupts binding to N-cadherin	Rhee *et al* (2007)
	Y654, Y670	Met (?)	Signalling, HFG-mediated release from membrane	Zeng *et al* (2006)
Ubiquitylation	K19	SCF ubiquitin ligase	Degradation	Wu *et al* (2003)
	K666, K671	Siah-1	Degradation, block of signalling	Dimitrova *et al* (2010)
Acetylation	K49	CBP	Signalling, enhancement of target specific β-catenin transcription *(c-myc)*	Wolf *et al* (2002)
	K345	p300	Signalling, increases binding of TCF, reduces binding to AR	Lévy *et al* (2004)
Glycosylation	(?)	O-GlcNAc transferase	Reduces nuclear localization	Sayat *et al* (2008)

Valenta et al. EMBO J 2012

B

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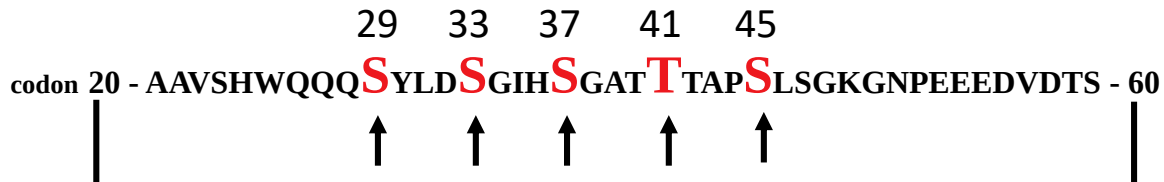
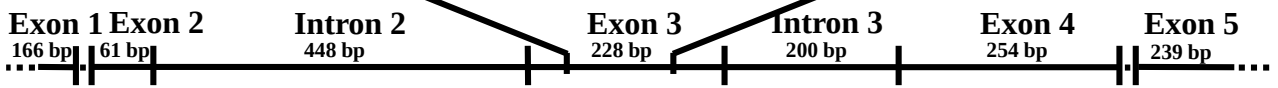
Valenta et al. EMBO J 2012

β-catenin phosphorylation sites

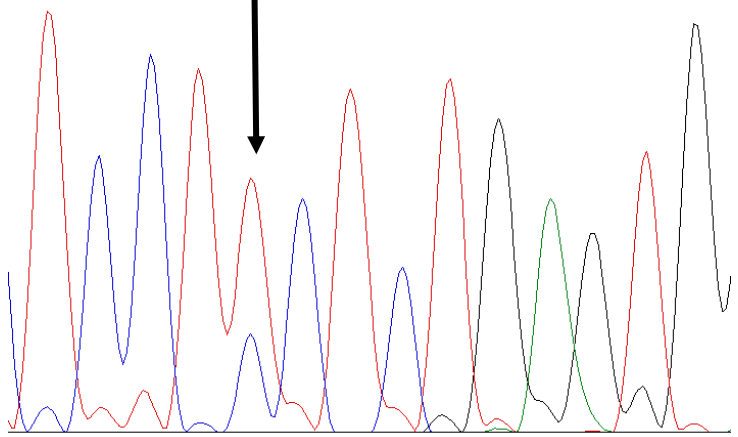


Amino acid sequence
S = serin
T= threonine



DNA sequence of the *CTNNB1* gene

T C T Wild type
C C T mutant sequence

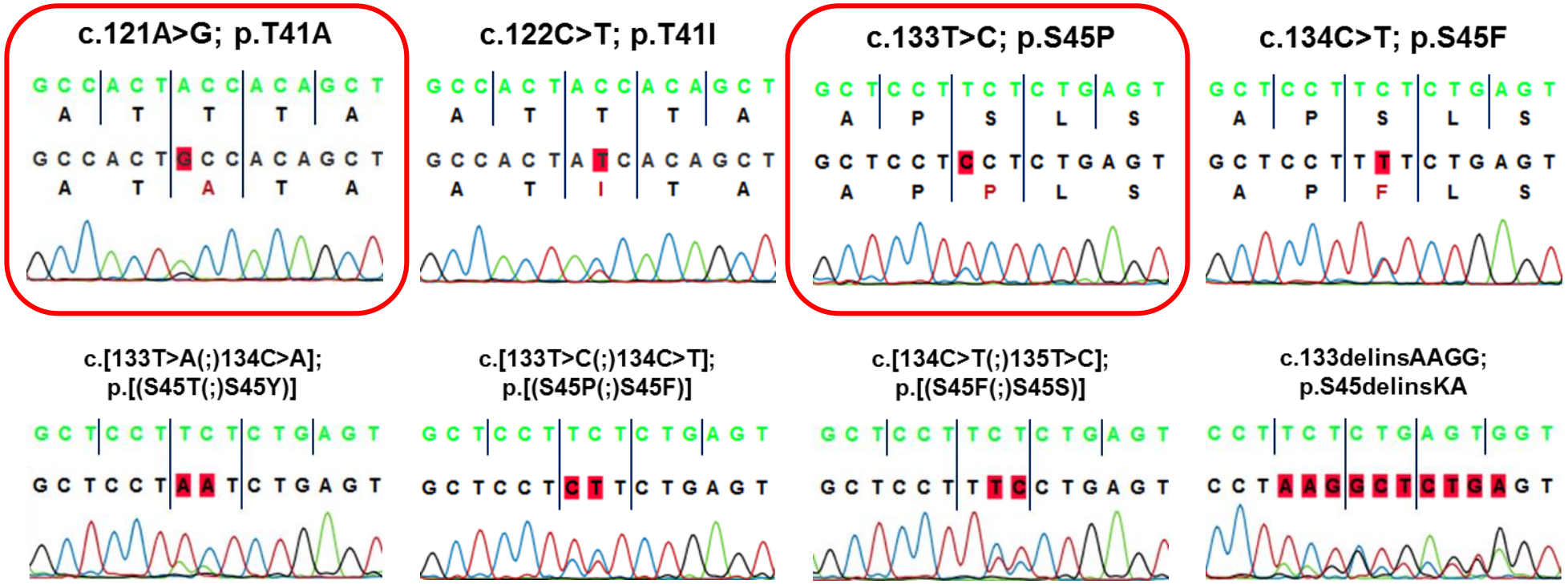


Point mutation in codon 45 (Ser-45Pro)

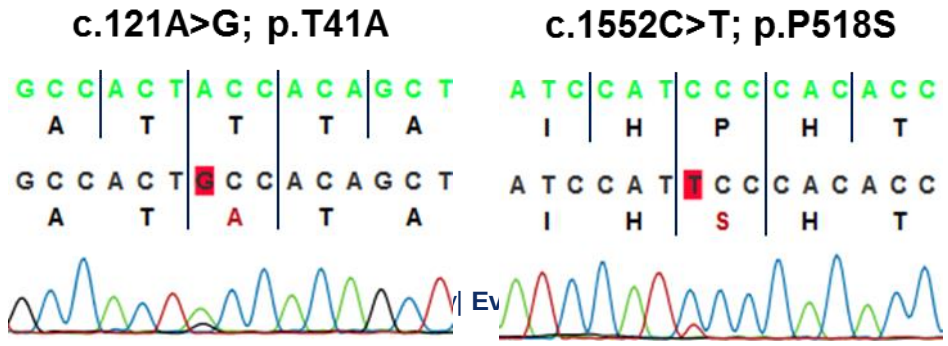
without phosphorylation
no degradation
-> ß-catenin accumulates!

Courtesy of Arend Koch

CTNNB1 (cadherin-associated protein beta) (exon 3)



B. cKIT (exon 10)

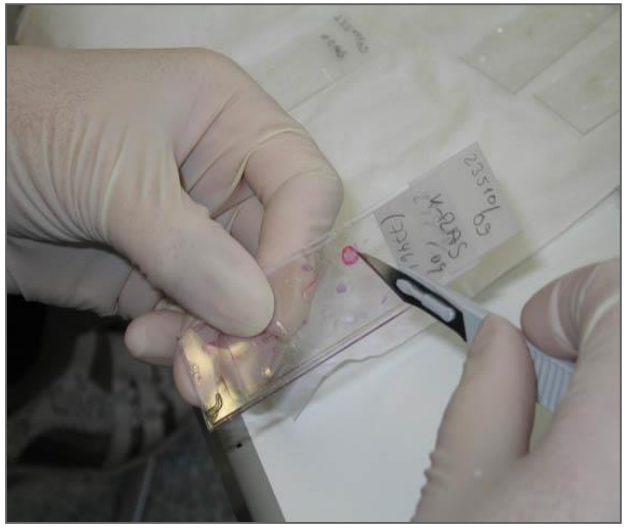


- in a subgroup of fibromatoses more complex *CTNNB1* mutations occur
- additional mutations in *KIT* exon 10 are observed in single cases

How to perform mutation analysis?
and
What is the relevance of mutation analysis?

Mutation analysis in desmoid fibromatosis
Technical aspects:

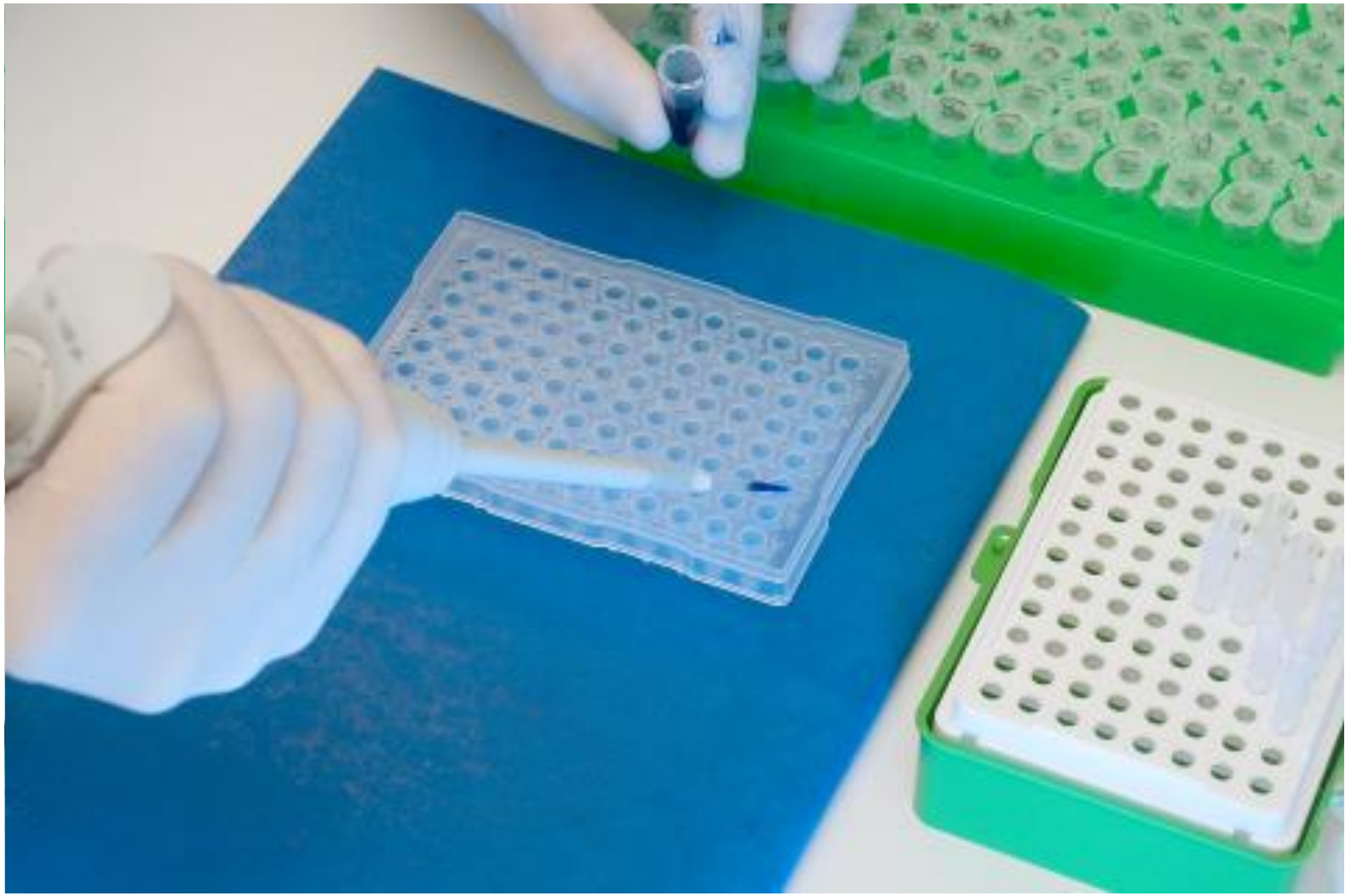
identification and quantification
of tumor content in the slide



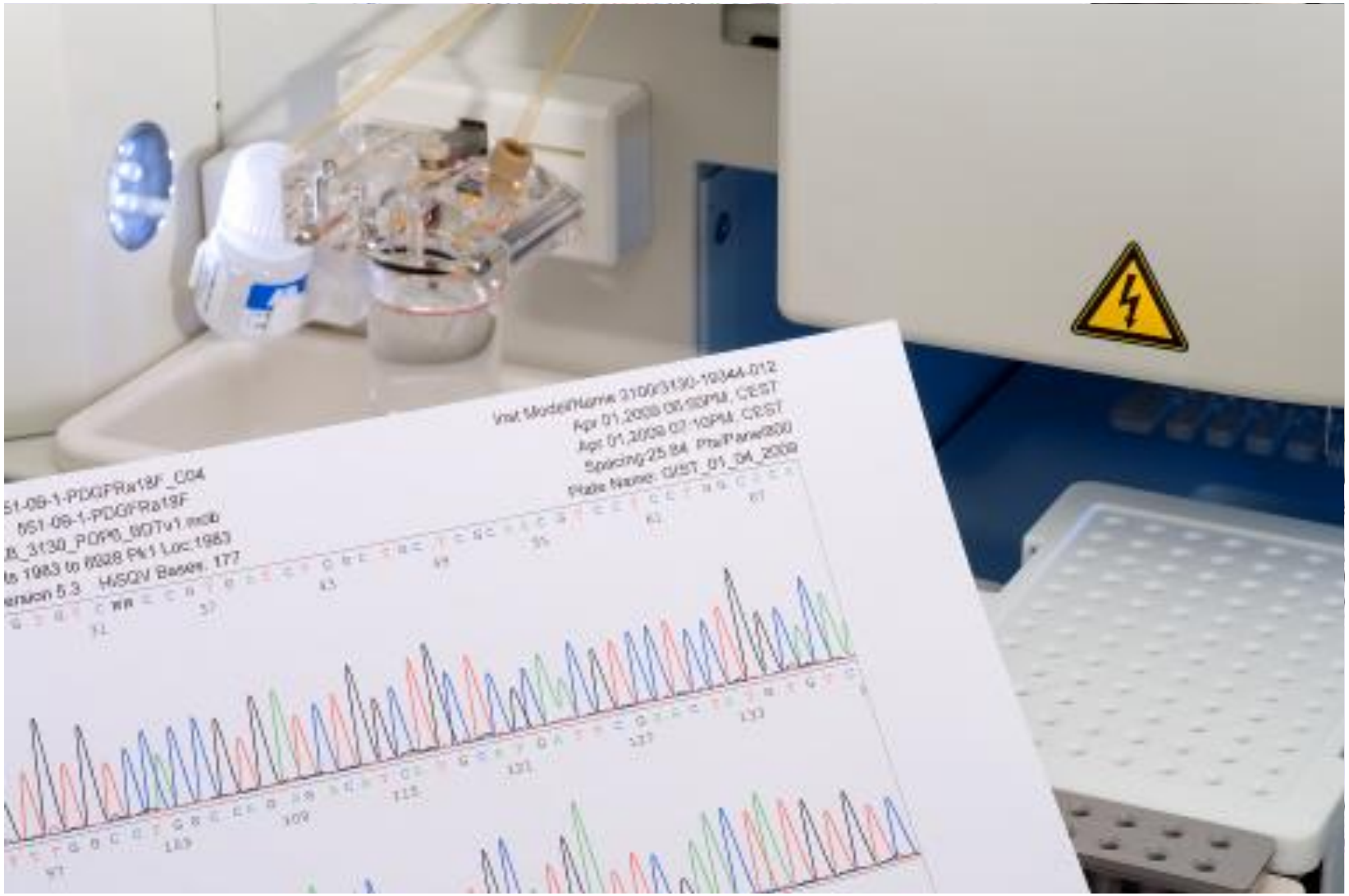
macrodissection

deparaffinisation

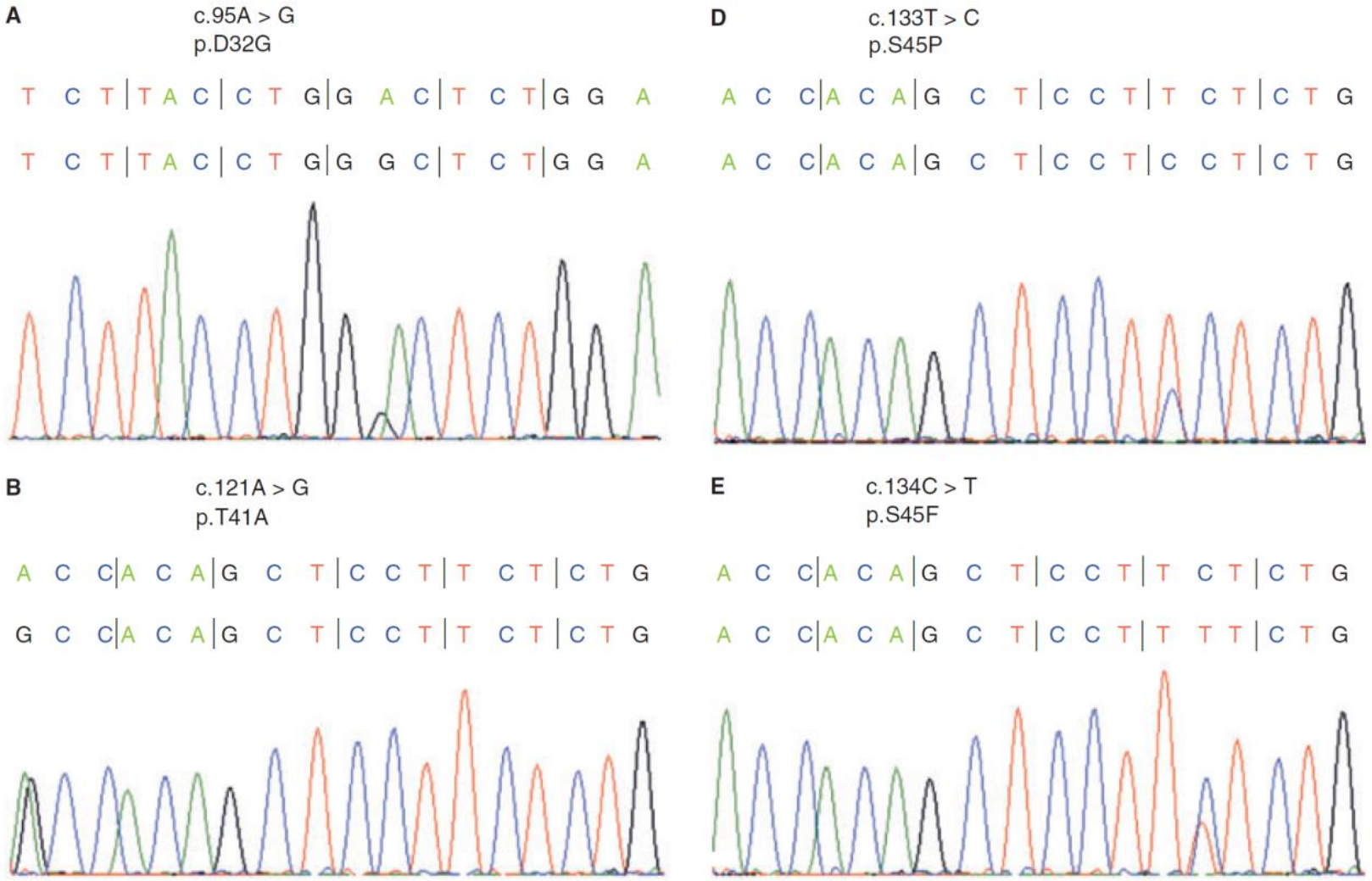
Mutation analysis in desmoid fibromatosis



Sanger Sequencing

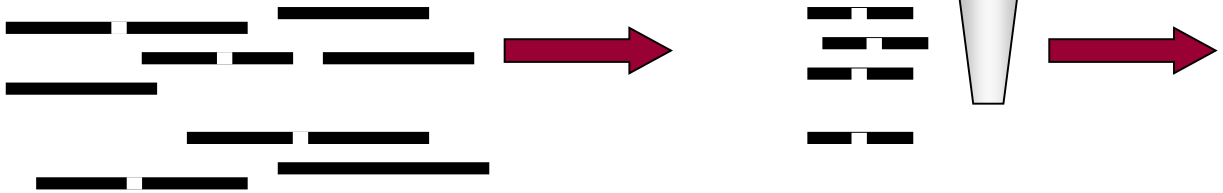


Sanger sequencing in desmoid fibromatosis



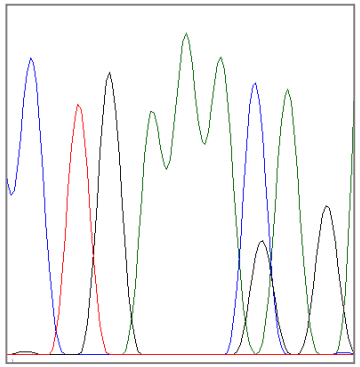
Next generation sequencing (NGS): principle

1st Generation Sequencing



DNA population

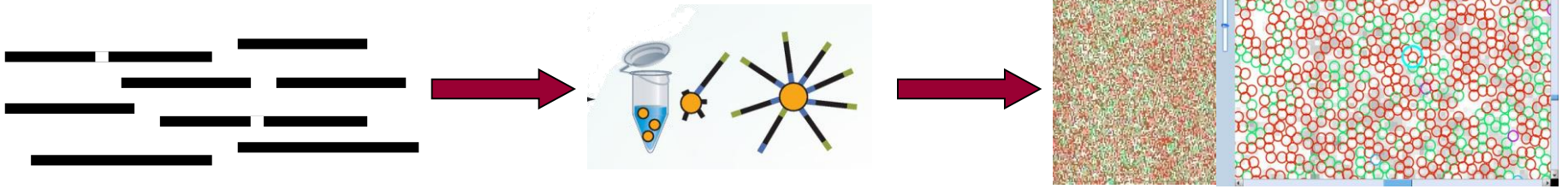
Simultaneous amplification



Simultaneous sequencing of the amplicon population

Next Generation Sequencing

DNA population

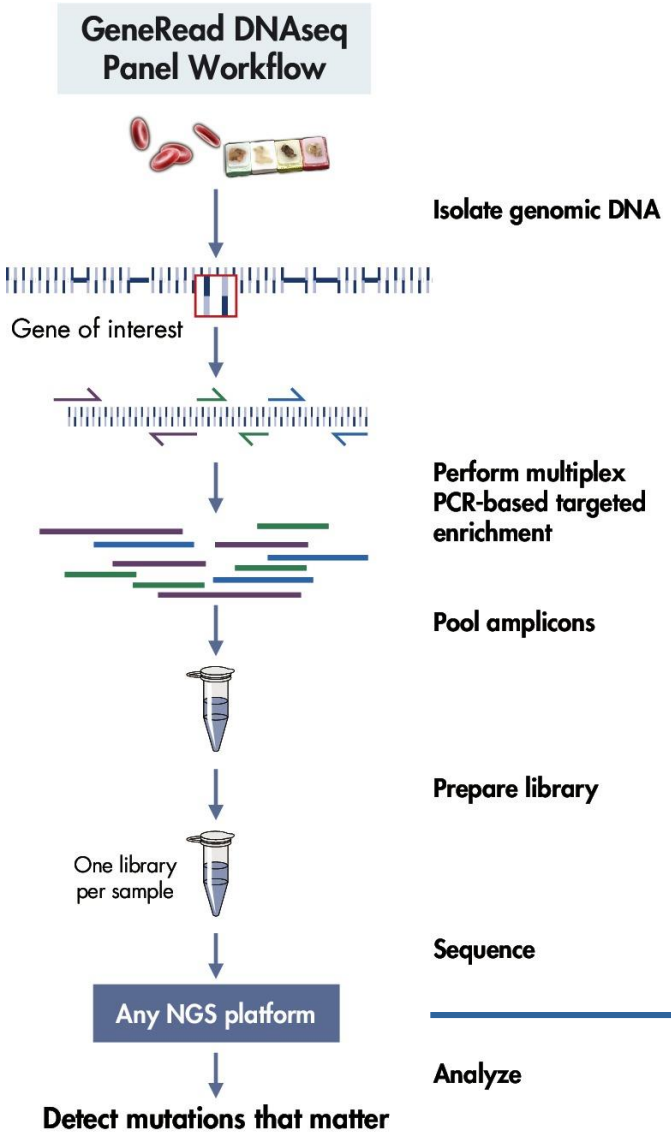


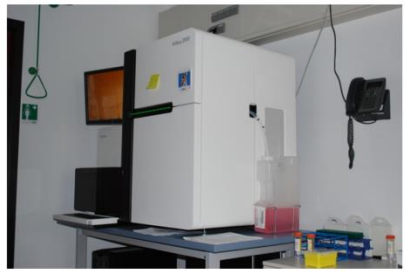
Adaptor ligation (Library)

clonal amplification of single molecules

Parallel sequencing of the amplicon clones

Next Generation Sequencing - Workflow

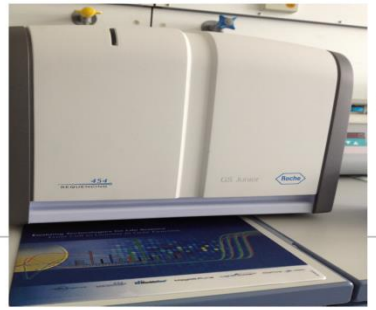




Illumina HiSeq + MiSeq

Ion Torrent PGM

Roche GS FLX



Roche 454 Junior

18

Seite 18

MiSeq - Illumina

β-Catenin (*CTNNB1*) mutations and clinicopathological features of mesenteric desmoid-type fibromatosis

Table 1. Baseline characteristics of patients in our cohort

Localization	*n*	Tumour size (mm)	Age at onset (years)	Sex (male/female)
Desmoid-type fibromatosis	84	106 (20–300)	44.9 (8–87)	37/47
Extra-abdominal	13	73 (25–140)	33.0 (8–77)	5/8
Abdominal wall	15	120 (30–300)	35.5 (19–64)	1/14
Mesenteric	56	109 (20–220)	50.0 (17–87)	31/25
Retroperitoneal fibrosis	11	49 (15–82)	52.9 (29–80)	5/6

Huss S, Nehles J, Binot E, Wardelmann E, Mittler J, Kleine M A, Künstlinger H, Hartmann W, Hohenberger P, Merkelbach-Bruse S, Buettner R & Schildhaus H-U
(2013) *Histopathology* **62**, 294–304

Table 2. Diagnostic criteria for mesenteric desmoid-type fibromatosis

Macroscopic findings	Firmish white mass
	Often macroscopically circumscribed
	Originates from mesentery but may infiltrate entire gut wall
Microscopic findings	Uniform bland spindle cells
	Stellate cells with hypochromatic nuclei
	Infiltrates surrounding soft tissue (might be absent)
	Fine fibrillar collagen around spindle cells in more cellular areas
	Frequent keloid-like collagen deposits
	Thin-walled dilated blood vessels
	Paucicellular perivascular spaces
Immunohistochemistry	Vimentin+
	SMA+/−
	Nuclear β-catenin+
Molecular analysis	*APC* mutations (germline, rare)
	CTNNB1 mutations (somatic, >80%)
Most frequent differential diagnoses	Dedifferentiated liposarcoma
	MDM2/CDK4 overexpression/amplification
	GIST (spindle cell phenotype)
	KIT+; CD34+; DOG-1+
	Typically originates from muscularis propria of stomach, small bowel, or rectum, but may extend into mesentery
	Retroperitoneal fibrosis (Ormond's disease)
	Hyalinized collagen
	Lymphoplasmacytic infiltrate
	Nuclear β-catenin–

CDK4, Cyclin-dependent kinase 4; GIST, gastrointestinal stromal tumour; SMA, smooth muscle actin.

Huss S, Nehles J, Binot E, Wardelmann E, Mittler J, Kleine M A, Künstlinger H, Hartmann W, Hohenberger P, Merkelbach-Bruse S, Buettner R & Schildhaus H-U
(2013) *Histopathology* **62**, 294–304

β-Catenin (*CTNNB1*) mutations and clinicopathological features of mesenteric desmoid-type fibromatosis

Table 3. *CTNNB1* mutational status of patients with desmoid-type fibromatosis and retroperitoneal fibrosis

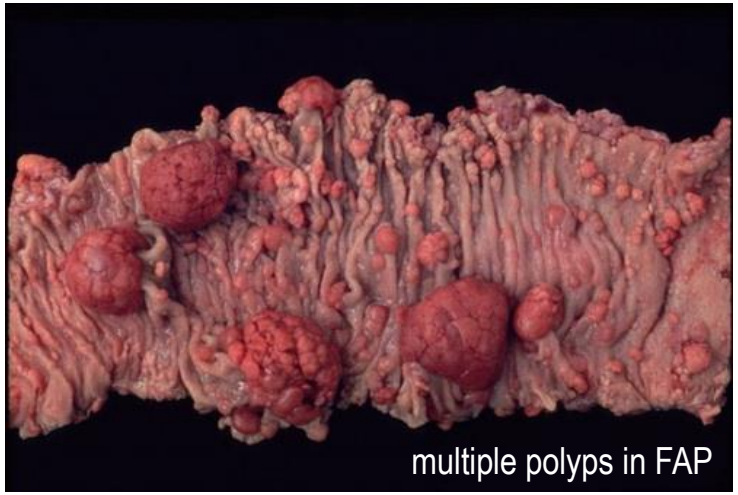
		CTNNB1 mutational status, *n* (%)						
Localization	*n*	WT [cases associated with FAP in square brackets]	D32G	T41A	S45F	S45P	S45C	Delins
Desmoid-type fibromatosis	84	13 (15.5) [5 (6.0)]	1 (1.2)	58 (69.0)	5 (6.0)	5 (6.0)	1 (1.2)	1 (1.2)*
Extra-abdominal	13	2 (15.4) [1 (7.7)]	0	7 (53.8)	2 (15.4)	2 (15.4)	0	0
Abdominal wall	15	6 (40.0) [4 (26.7)]	1 (6.7)	6 (40.0)	1 (6.7)	1 (6.7)	0	0
Mesenteric	56	5 (8.9) [0]	0	45 (80.4)	2 (3.6)	2 (3.6)	1 (1.8)	1 (1.8)*
Retroperitoneal fibrosis	11	0 [0]	0	0	0	0	0	0

FAP, Familial adenomatous polyposis coli (Gardner's syndrome); WT, wild type.
*p.T42_K49delinsQ.

Huss S, Nehles J, Binot E, Wardelmann E, Mittler J, Kleine M A, Künstlinger H, Hartmann W, Hohenberger P, Merkelbach-Bruse S, Buettner R & Schildhaus H-U
(2013) *Histopathology* **62**, 294–304

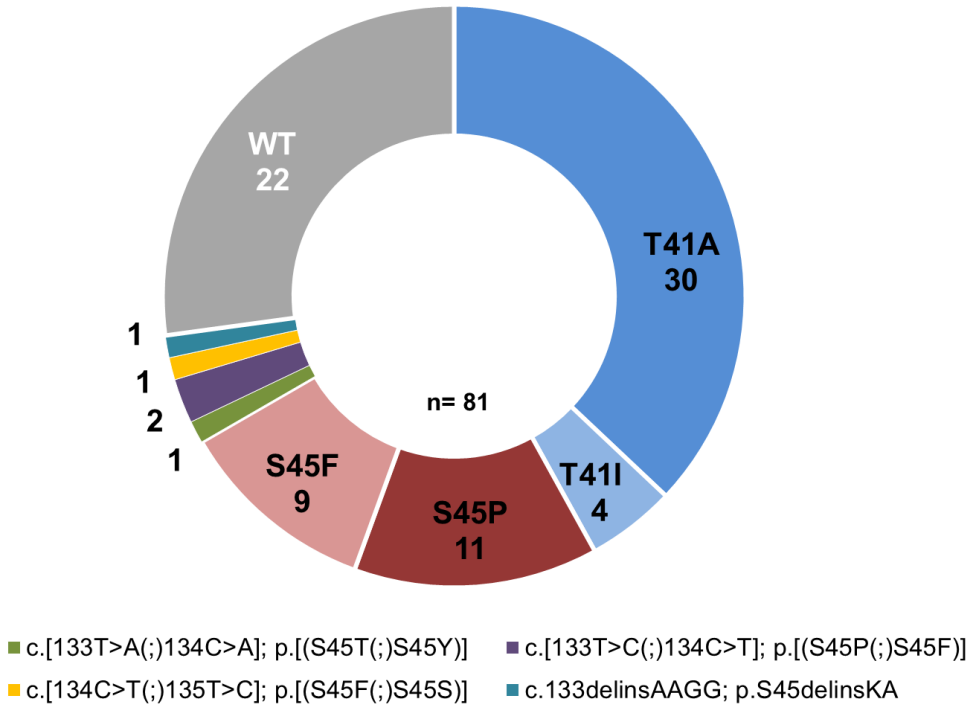
What about patients without *CTNNB1* mutations?

- the majority of them has an APC mutation, mostly in a hereditary background
- APC germline mutations lead to familial adenomatous polyposis (FAP)
- esp. in young patients without *CTNNB1* mutation FAP should be considered and patients should get coloscopy to exclude such a possibility
- a small minority of patients has sporadic APC mutations
- very few patients have alterations in other parts of the Wnt pathway


multiple polyps in FAP

Correlation between ß-catenin expression and *CTNNB1* mutation

CTNNB1 mutation		Nuclear ß-catenin expression	
		Positive	Negative
D32G		1	0
T41A		54	4
S45F		5	0
S45P		4	1
S45C		1	0
T42_K49delinsQ		1	0
Wild type	Associated with FAP	3	2
	Not associated with FAP	8	0
Total		77	7

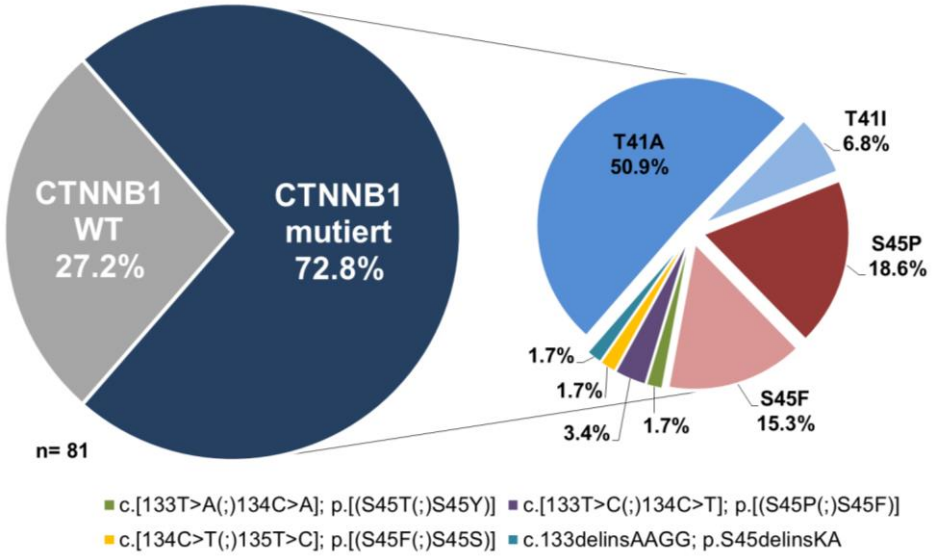


Huss, S. et al. Histopathology 2013

Most frequent mutational subtypes in *CTNNB1* in fibromatosis

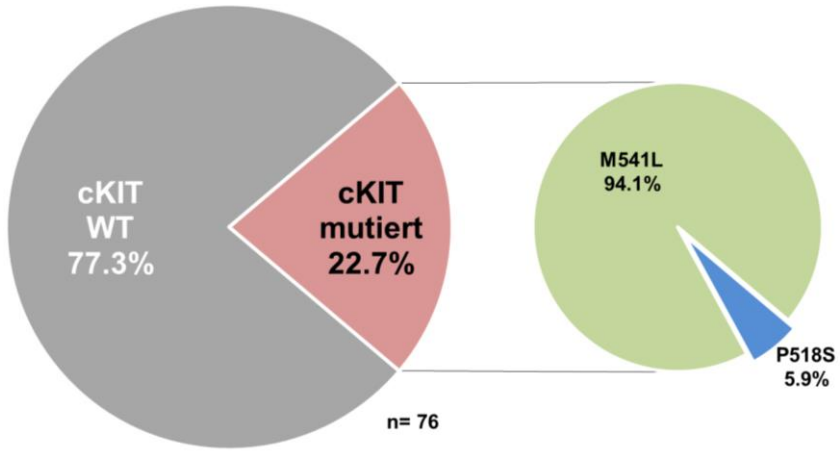
	n	%
WT	22	25,9
p.T41A	37	43,5
p.T41I	4	4,7
p.S45F	7	8,2
p.S45P	10	11,8
p.[(S45T(;)S45Y)]*	1	1,2
p.[(S45P(;)S45F)]*	2	2,4
p.[(S45F(;)S45S)]*	1	1,2
p.S45delinsKA	1	1,2
n(total)	85	100

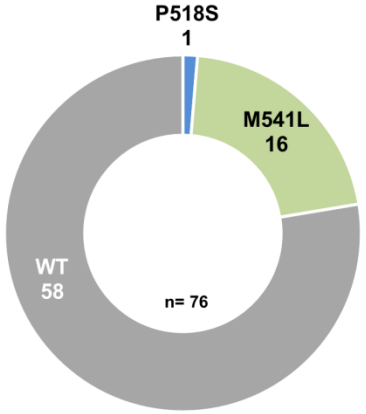
*double mutant

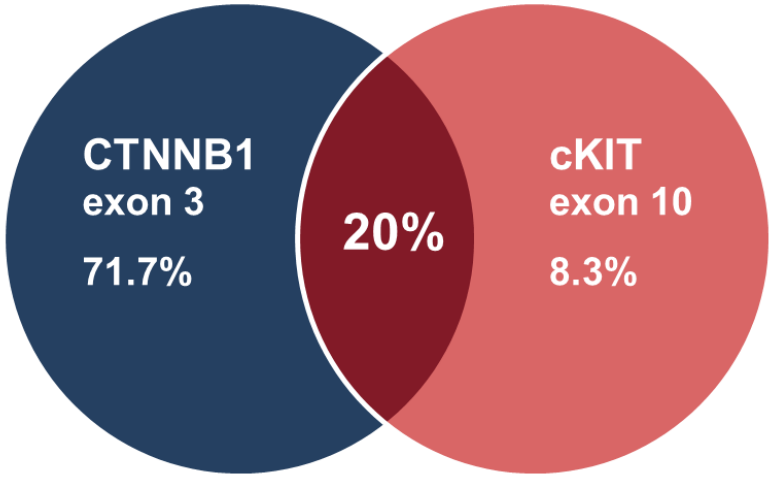


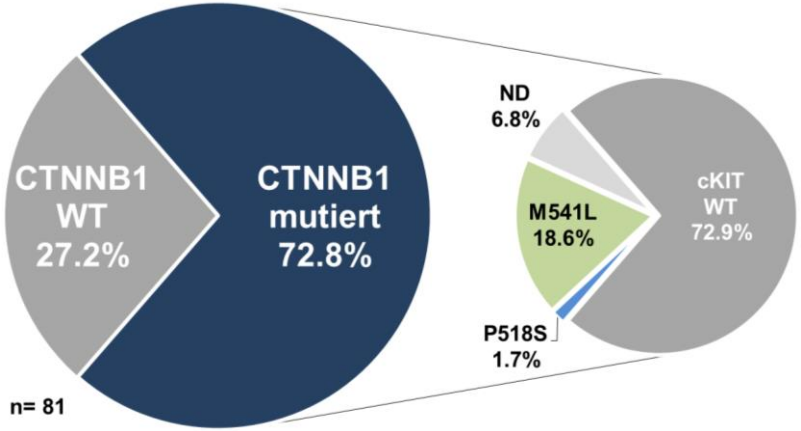
Huss, S. et al. Histopathology 2013

KIT exon 10 variants: Relevance for biological behavior or for response to treatment?









Huss, S. et al. Histopathology 2013

Impact of *KIT* exon 10 M541L allelic variant on the response to imatinib in aggressive fibromatosis: analysis of the desminib series by competitive allele specific Taqman PCR technology

BMC Cancer 2014

Armelle Dufresne[1,2*], Laurent Alberti[1], Mehdi Brahmi[1], Sarah Kabani[1], Héloïse Philippon[1], David Pérol[3] and Jean Yves Blay[1,2]

Table 2 Distribution of objective response observed at 6 months and 1 year according to *KIT* status

	KIT^{WT} (n = 22)	KIT^{L541} (n = 5)	Chi 2
Response at 6 months			
CR/PR	3	1	
SD	15	4	
PD	4	0	
			p = 0,57683407
Response at 1 year			
CR/PR	2	1	
SD	13	4	
PD	7	0	p = 0,31614938

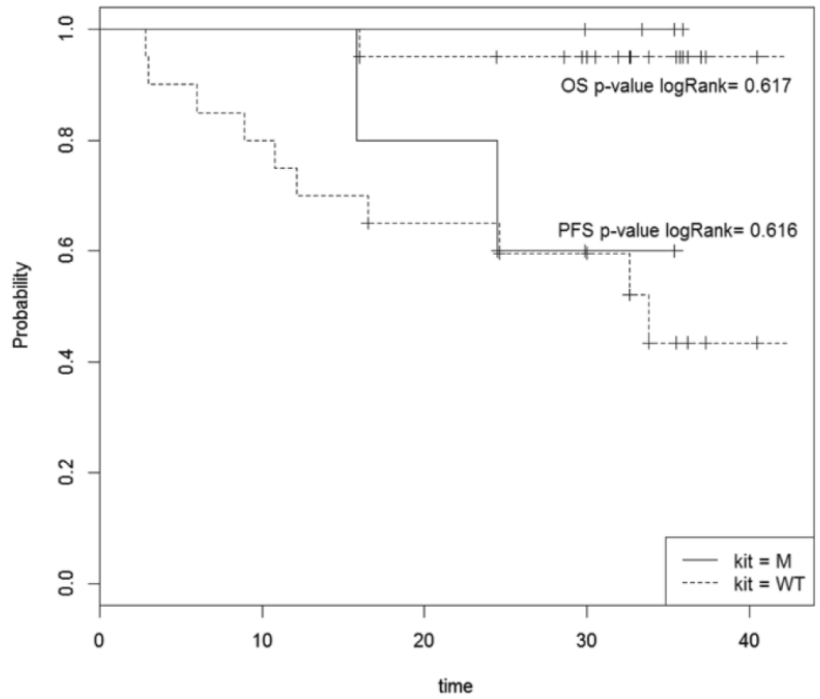


Figure 4 Log-rank analysis of progression-free survival (PFS) and overall survival (OS) for patients with (M) and without (WT) KIT^{L541} variant in phase II Desminib trial.

Prognostic relevance of the mutational subtype in CTNNB1 in fibromatosis

Sporadic desmoid-type fibromatosis: a stepwise approach to a non-metastasising neoplasm—a position paper from the Italian and the French Sarcoma Group

A. Gronchi[1*], C. Colombo[1], C. Le Péchoux[2], A. P. Dei Tos[3], A. Le Cesne[4], A. Marrari[5], N. Penel[6], G. Grignani[7], J. Y. Blay[8], P. G. Casali[5], E. Stoeckle[9], F. Gherlinzoni[10], P. Meeus[11], C. Mussi[12], F. Gouin[13], F. Duffaud[14], M. Fiore[1], S. Bonvalot[15] & on behalf of ISG and FSG Ann Oncol 2013

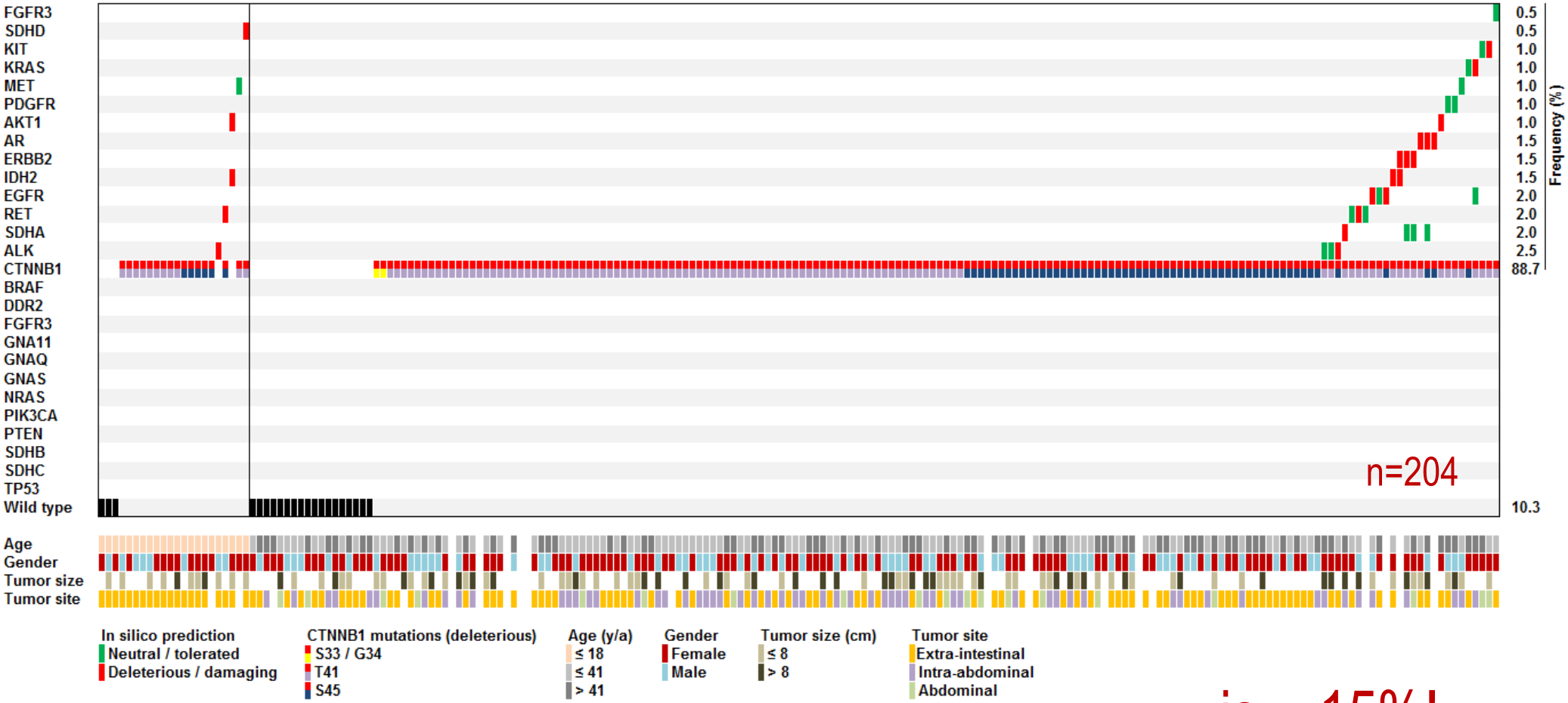
Prognostic Value of *CTNNB1* Gene Mutation in Primary Sporadic Aggressive Fibromatosis

Danique L. M. van Broekhoven, MD[1], Cornelis Verhoef, MD, PhD[1], Dirk J. Grünhagen, MD, PhD[1], Joost M. H. H. van Gorp, MD, PhD[2], Michael A. den Bakker, MD, PhD[3], John W. J. Hinrichs, PhD[4], Carmen M. A. de Voijs[4], and Thijs van Dalen, MD, PhD[5] Ann Surg Oncol 2015

Correlation of *CTNNB1* Mutation Status with Progression Arrest Rate in RECIST Progressive Desmoid-Type Fibromatosis Treated with Imatinib: Translational Research Results from a Phase 2 Study of the German Interdisciplinary Sarcoma Group (GISG-01)

Bernd Kasper, MD, PhD[1], Viktor Gruenwald, MD, PhD[2], Peter Reichardt, MD[3], Sebastian Bauer, MD, PhD[4], Peter Hohenberger, MD, PhD[1], and Florian Haller, MD, PhD[5] Ann Surg Oncol 2016

The frequency of additional mutations in fibromatosis…



…is ~ 15%!

Trautmann et al. submitted

UKM
Universitätsklinikum
Münster

Summary

- Desmoid-type fibromatosis can occur everywhere
- biological behavior is not predictable
- S45F mutations could be an indicator of an increased recurrence risk
- S45 mutations are associated with a higher progression arrest rate under imatinib compared to T41 mutations and wild type
- a subgroup of desmoid type-fibromatoses has additional allelic variants in *KIT* exon 10, probably polymorphisms with clinical relevance
- Additional mutations occur in 15% of cases
- Patients with *CTNNB1*-wildtype desmoids could be FAP patients and should get a coloscopy
- Treatment strategies are still diverse, including „watch and wait“

University Hospital Münster

Gerhard-Domagk-Institute of Pathology

KO.SAR. Kompetenznetz SARKOME

GISG German Interdisciplinary Sarcoma Group

PATHSARK Pathologienetzwerk Sarkome

EORTC European Organisation for Research and Treatment of Cancer

SARKOME.DE DEUTSCHE SARKOM STIFTUNG

Deutsche Krebshilfe Helfen. Forschen. Informieren.

Scandinavian Sarcoma Group 1979


Thank you for your attention