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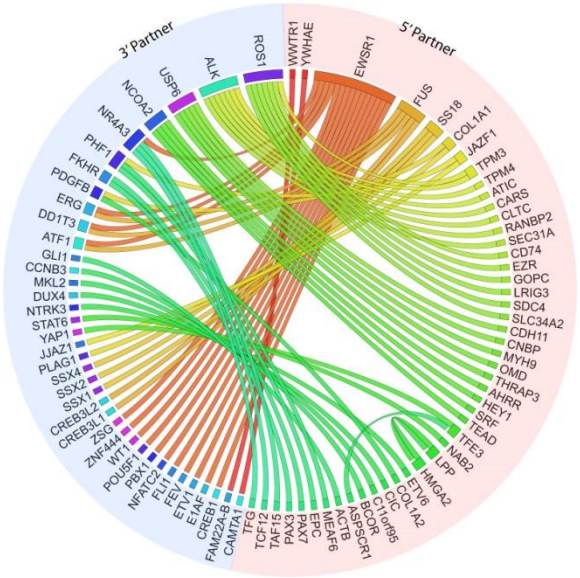
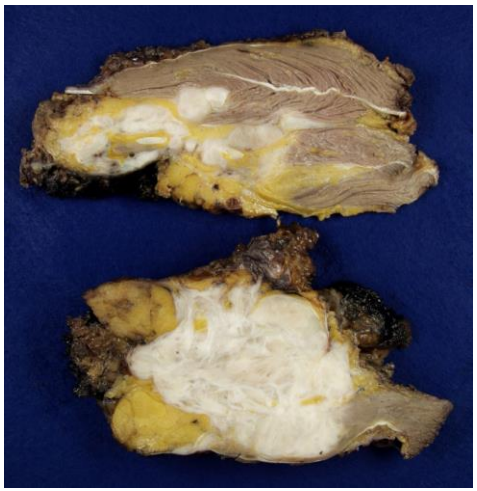
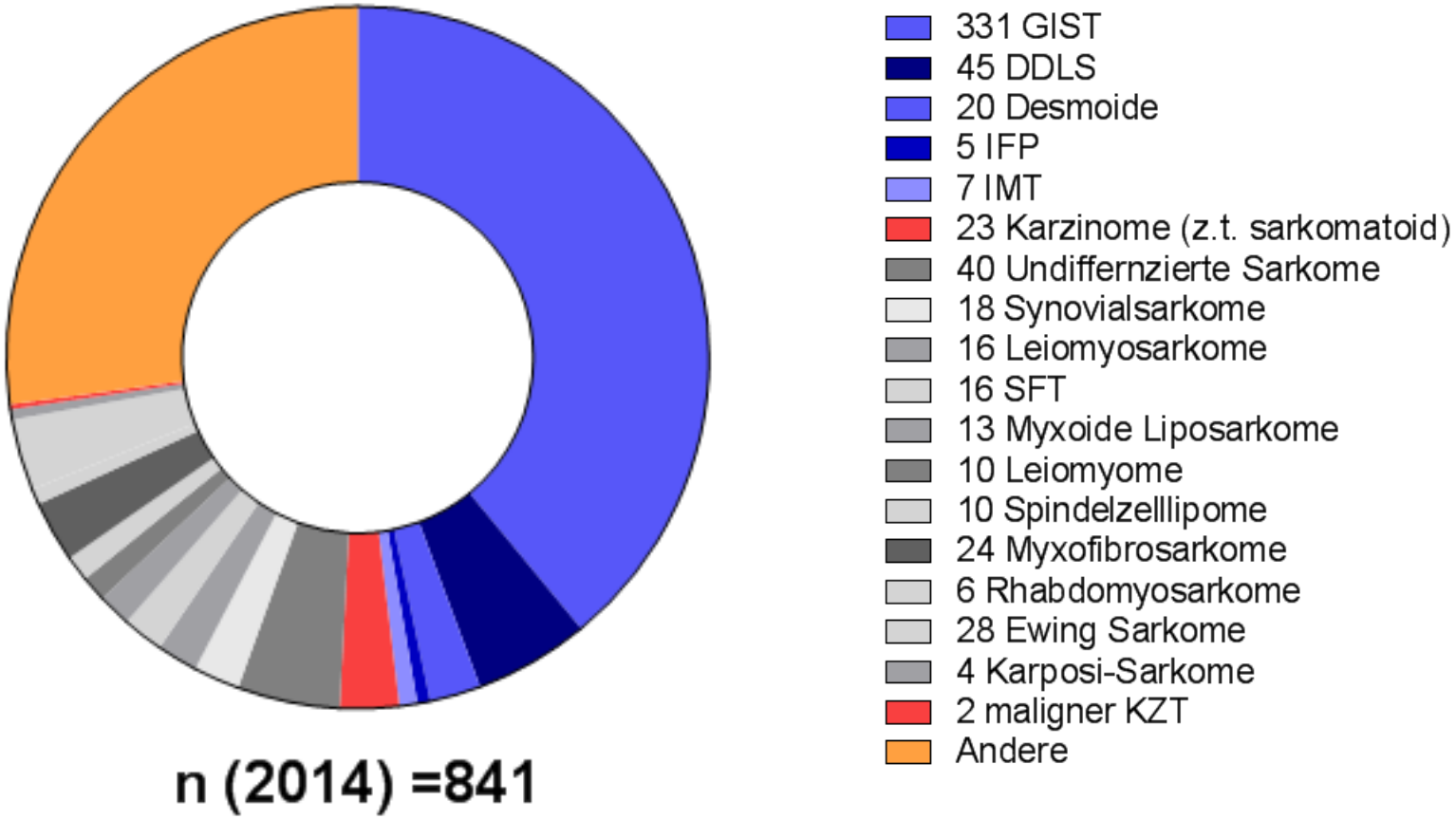




Biologie/Genetik der Desmoide: Die Rolle des Pathologen

13. Oktober 2020| **Eva Wardelmann, Gerhard-Domagk-Institut für Pathologie, Universitätsklinikum Münster**

Häufigkeit von Weichgewebstumoren in unserem Register (in 2014 n=841)



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Welche Rolle spielt der Pathologe für Patienten mit Desmoidfibromatose?

Begriffsklärung

- Fibromatose
- Desmoid
- Desmoidfibromatose
- Superfizielle Fibromatose
- Morbus Dupuytren
- Morbus Ledderhose
- …

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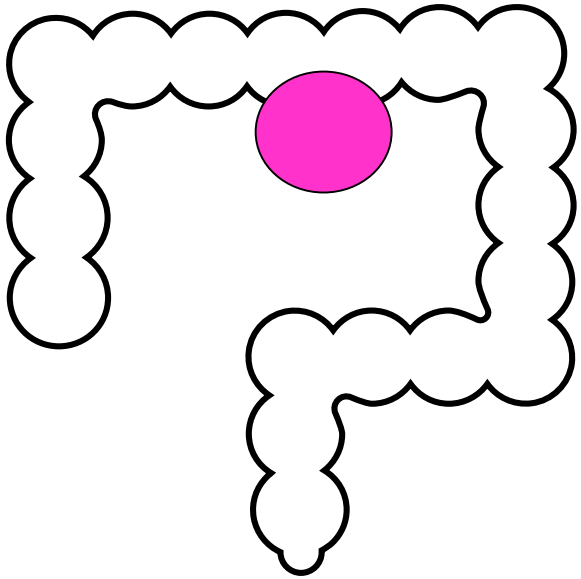
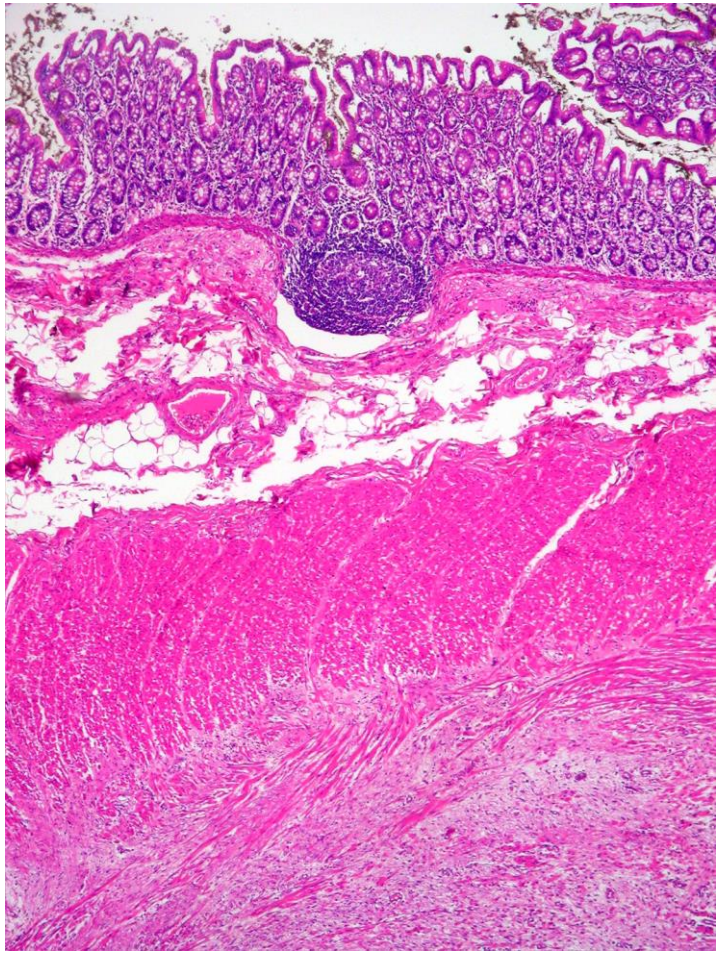
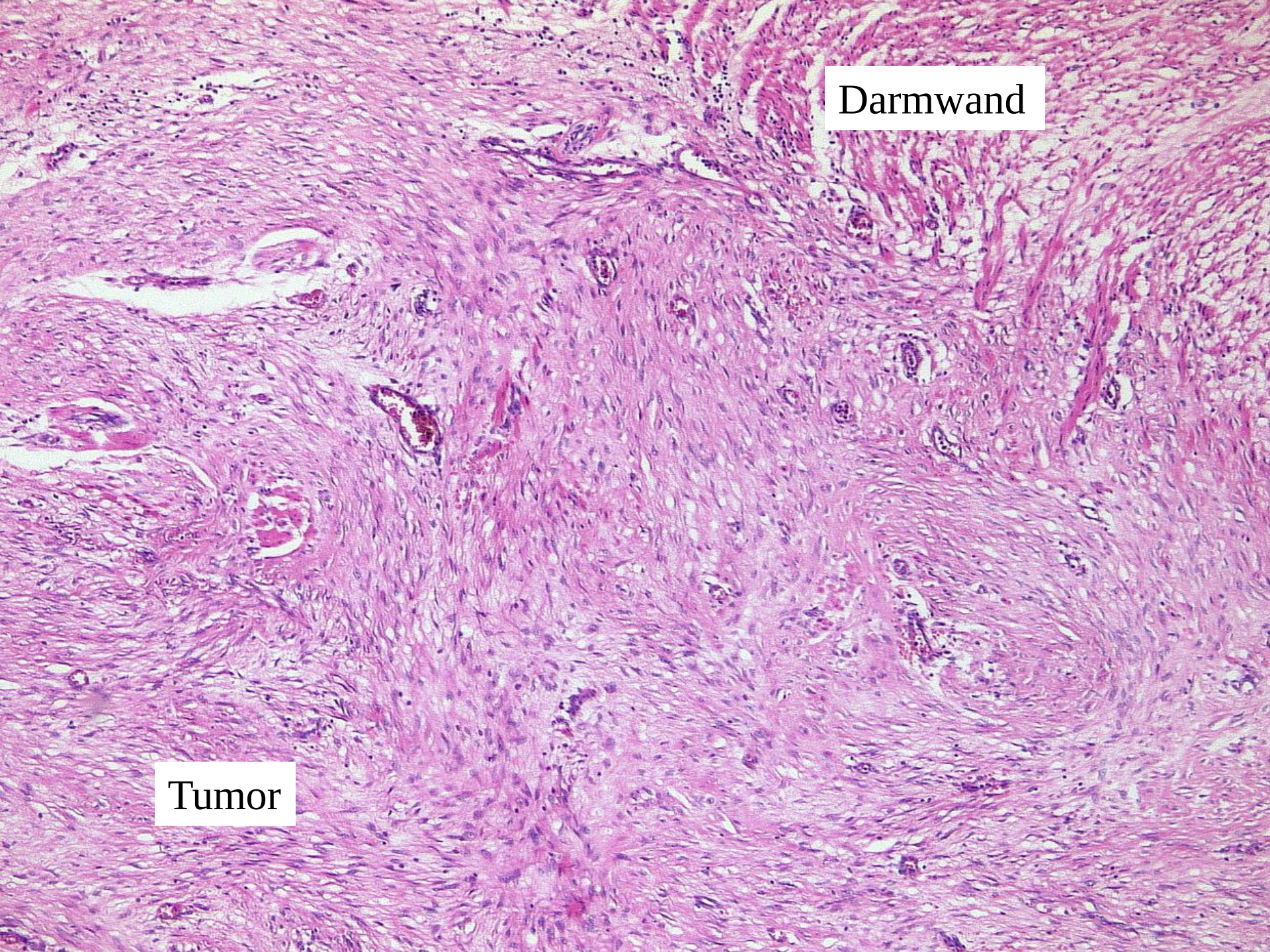
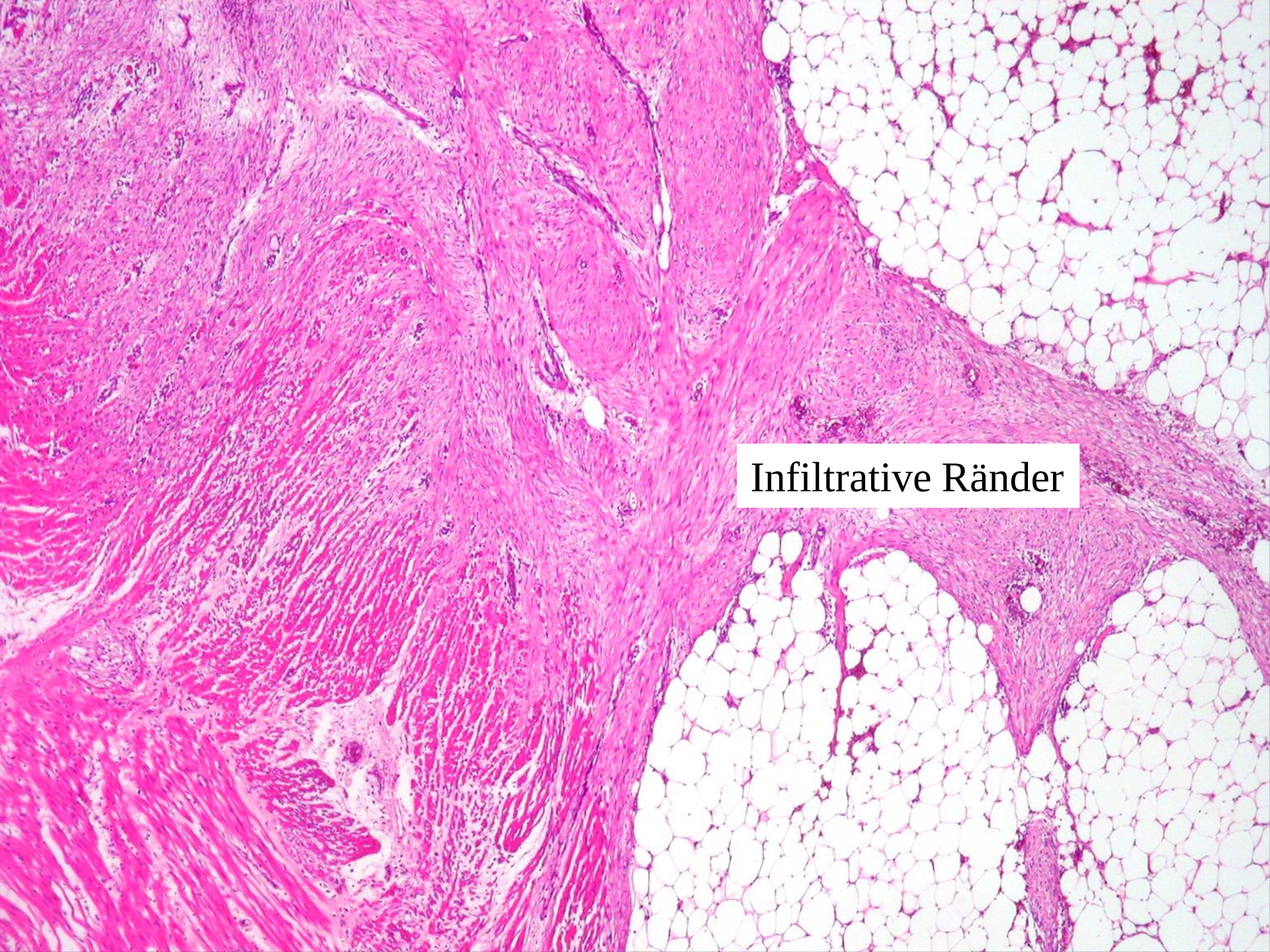
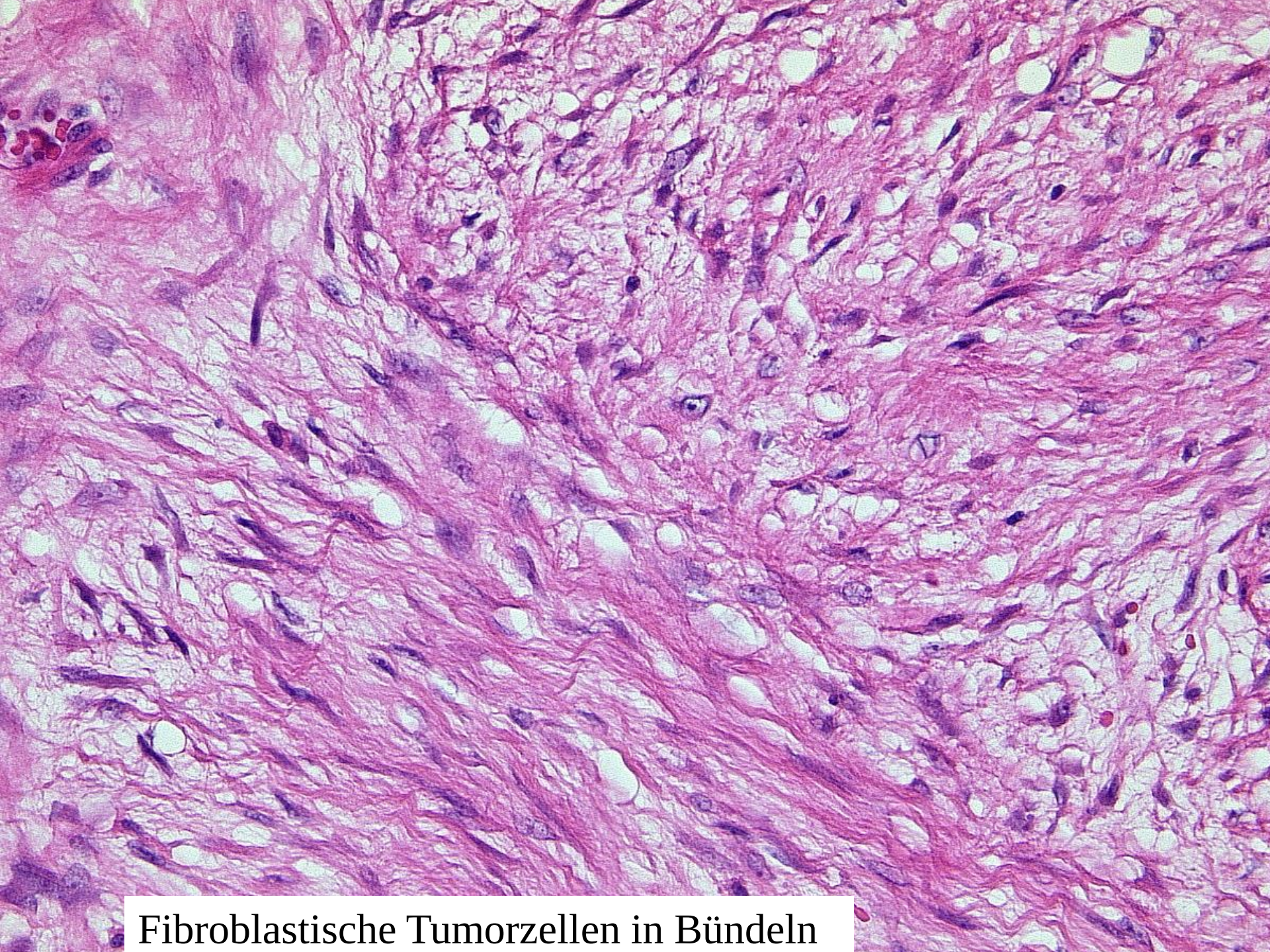
39 Jahre alte Frau mit einem 6 cm großen Knoten am Colon transversum



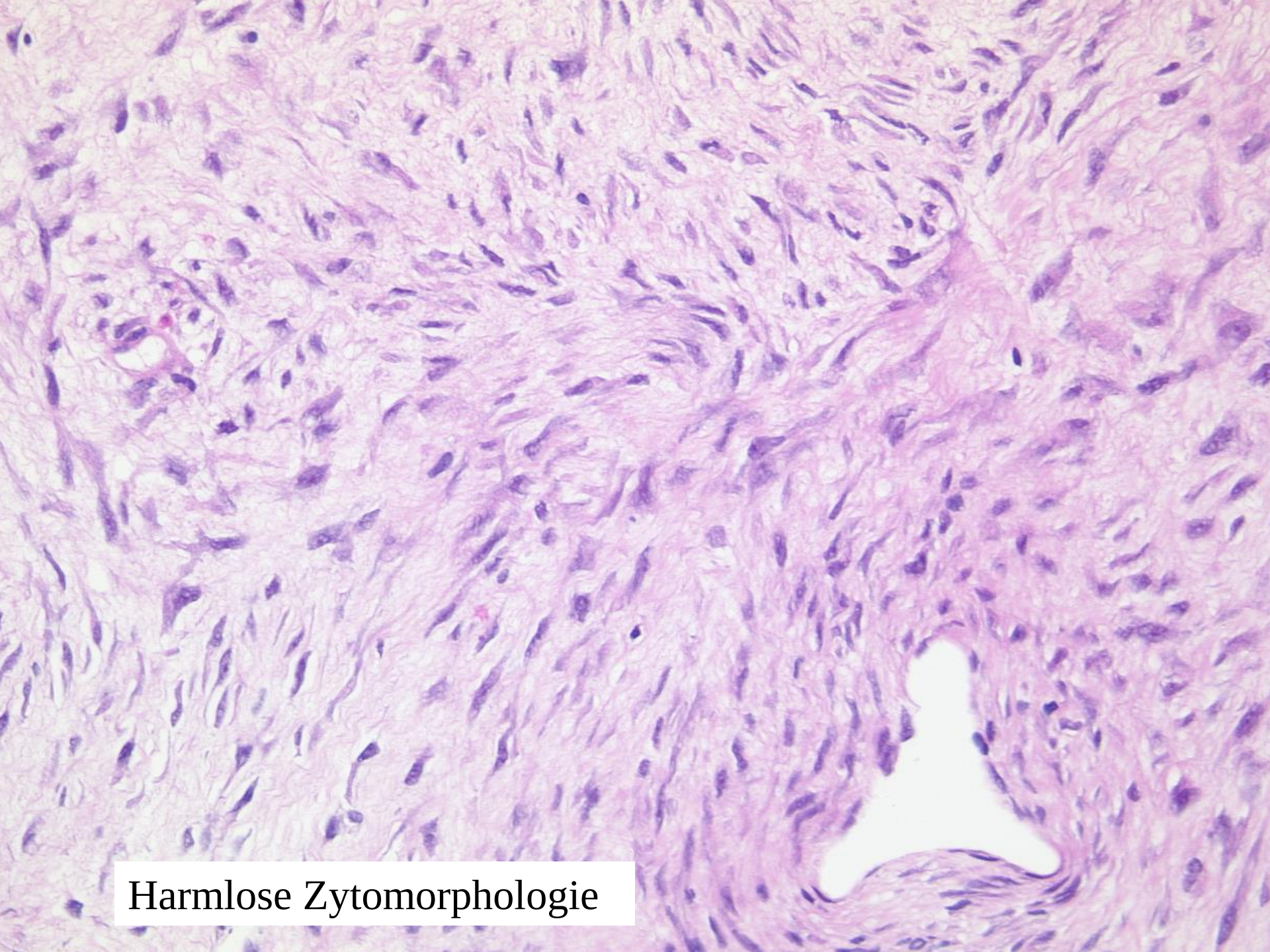


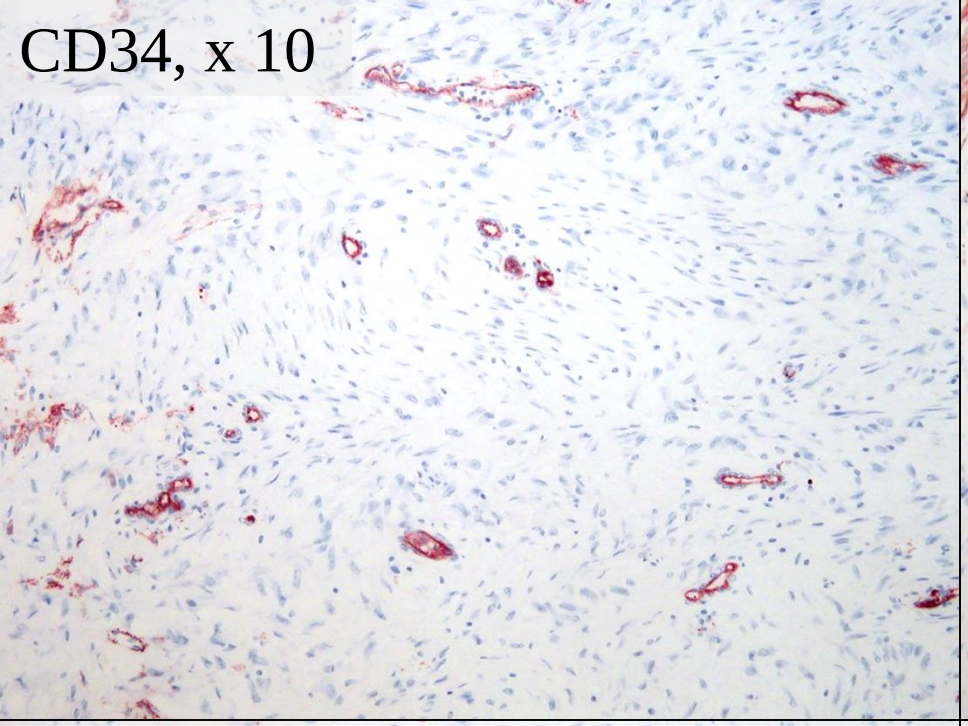

Darmwand
Tumor

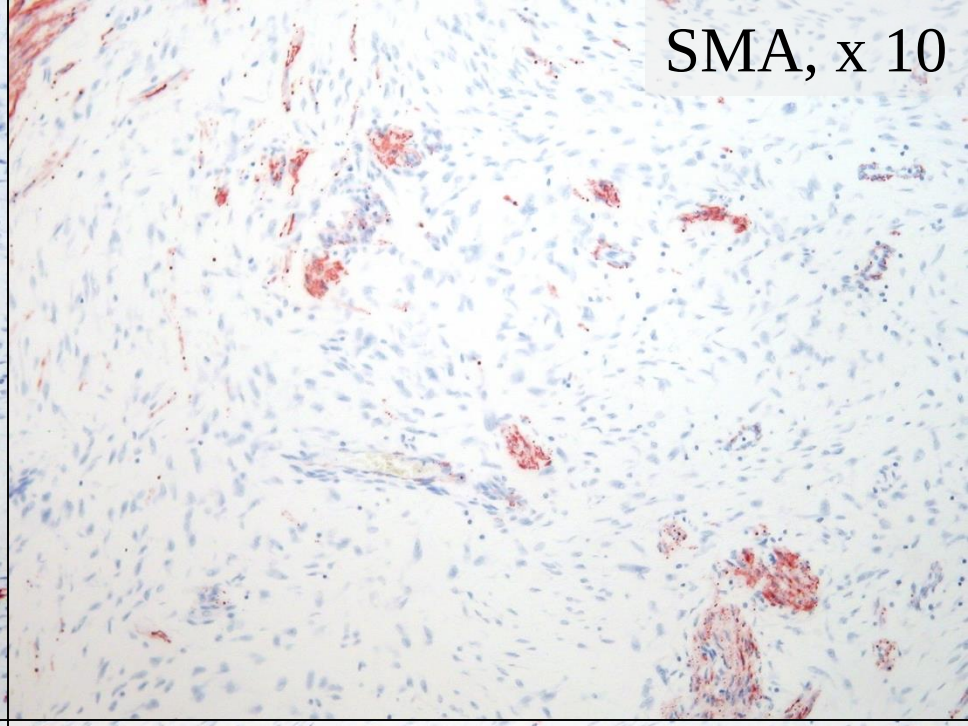

Infiltrative Ränder

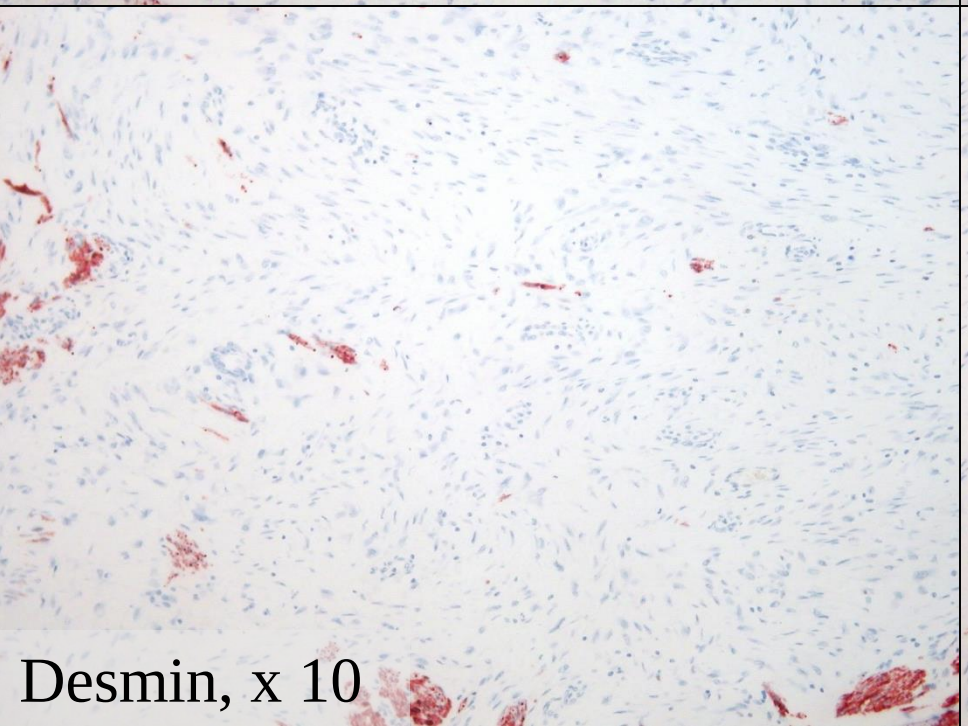


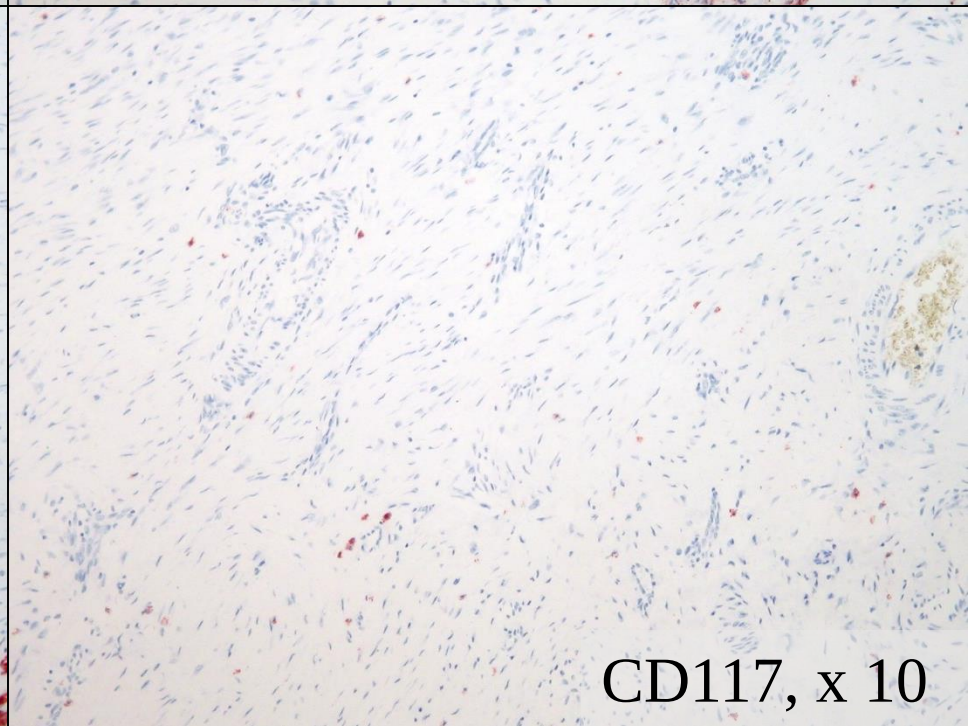
Fibroblastische Tumorzellen in Bündeln

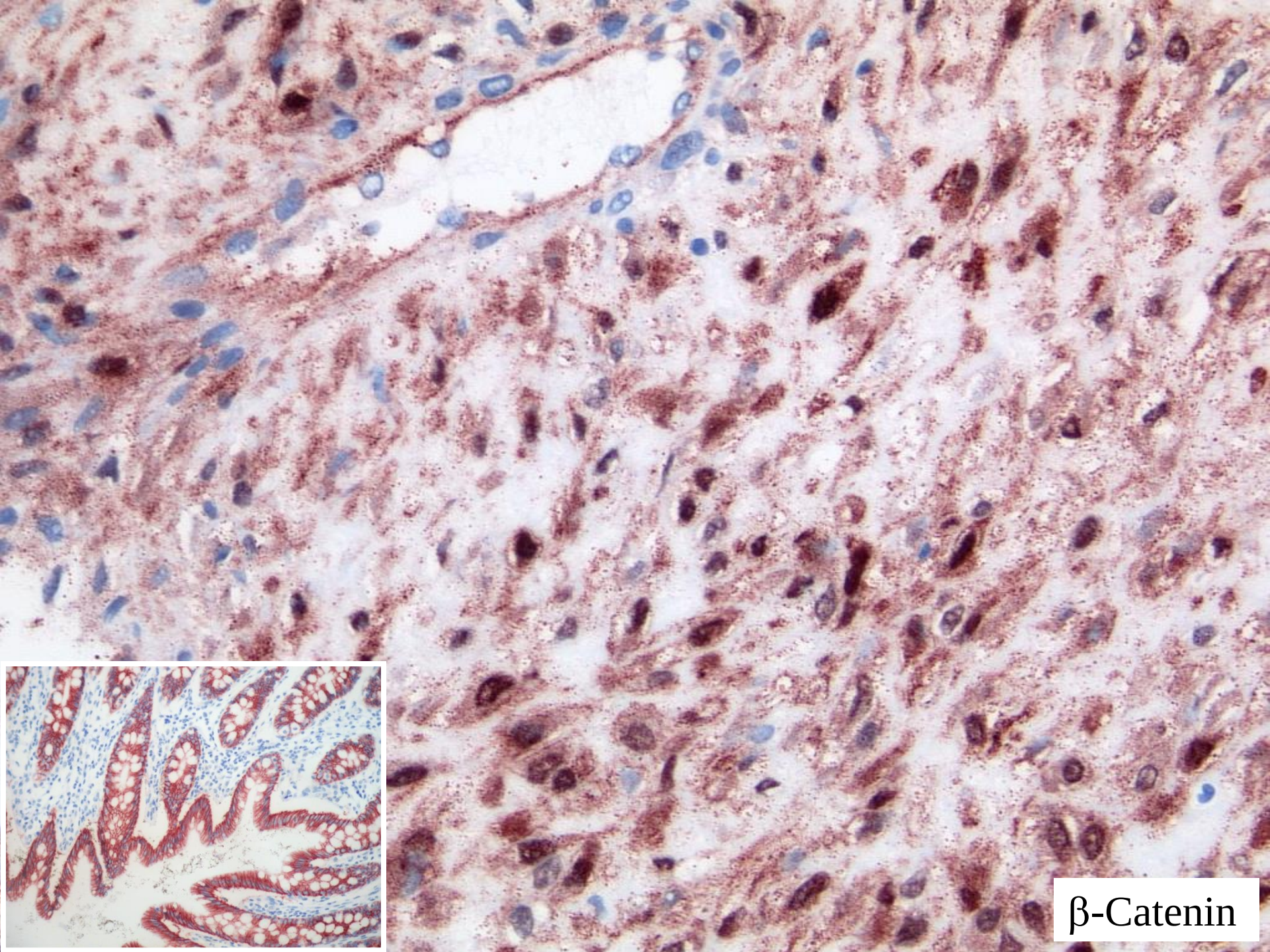

Harmlose Zytomorphologie


CD34, x 10

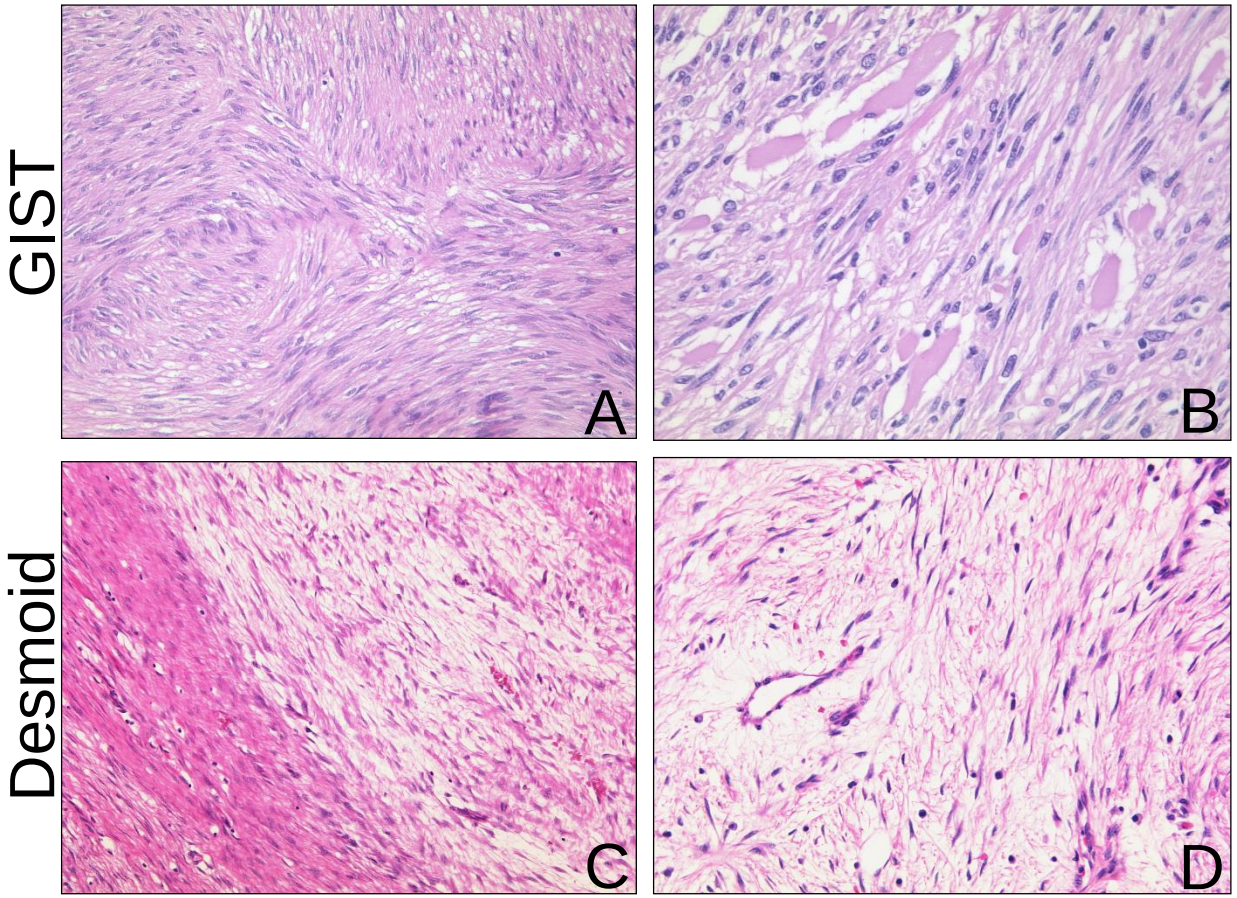

SMA, x 10


Desmin, x 10


CD117, x 10


β-Catenin

Morphologische Ähnlichkeit zu gastrointestinalen Stromatumoren (GIST)



Wichtige Differentialdiagnosen

Dedifferenziertes Liposarkom

MDM2/CDK4 Überexpression/Amplifikation

GIST (spindelzellig)

KIT+; CD34+; DOG-1+;

Typischweise mit der Lamina muscularis propria des tubulären GI Trakts ver-

Bunden, wölbt sich oft ins Mesenterium oder die Bauchhöhle vor

Retroperitoneale Fibrose (Morbus Ormond)

Hyalinisiertes Kollagen

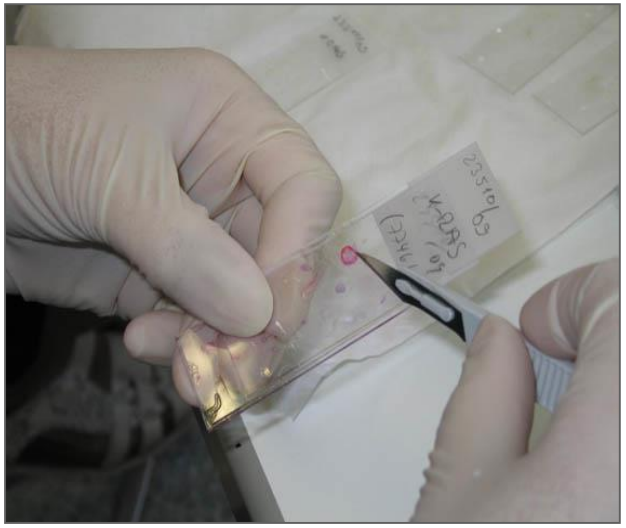
Lymphoplasmocytäres Entzüdnungsinfiltrat, IgG4-positive Plasmazellen

Keine nukleäre ß-Cateninexpression

Huss, S. et al. Histopathology 2013

Mutationsanalyse bei Desmoidfibromatose
Technische Aspekte:

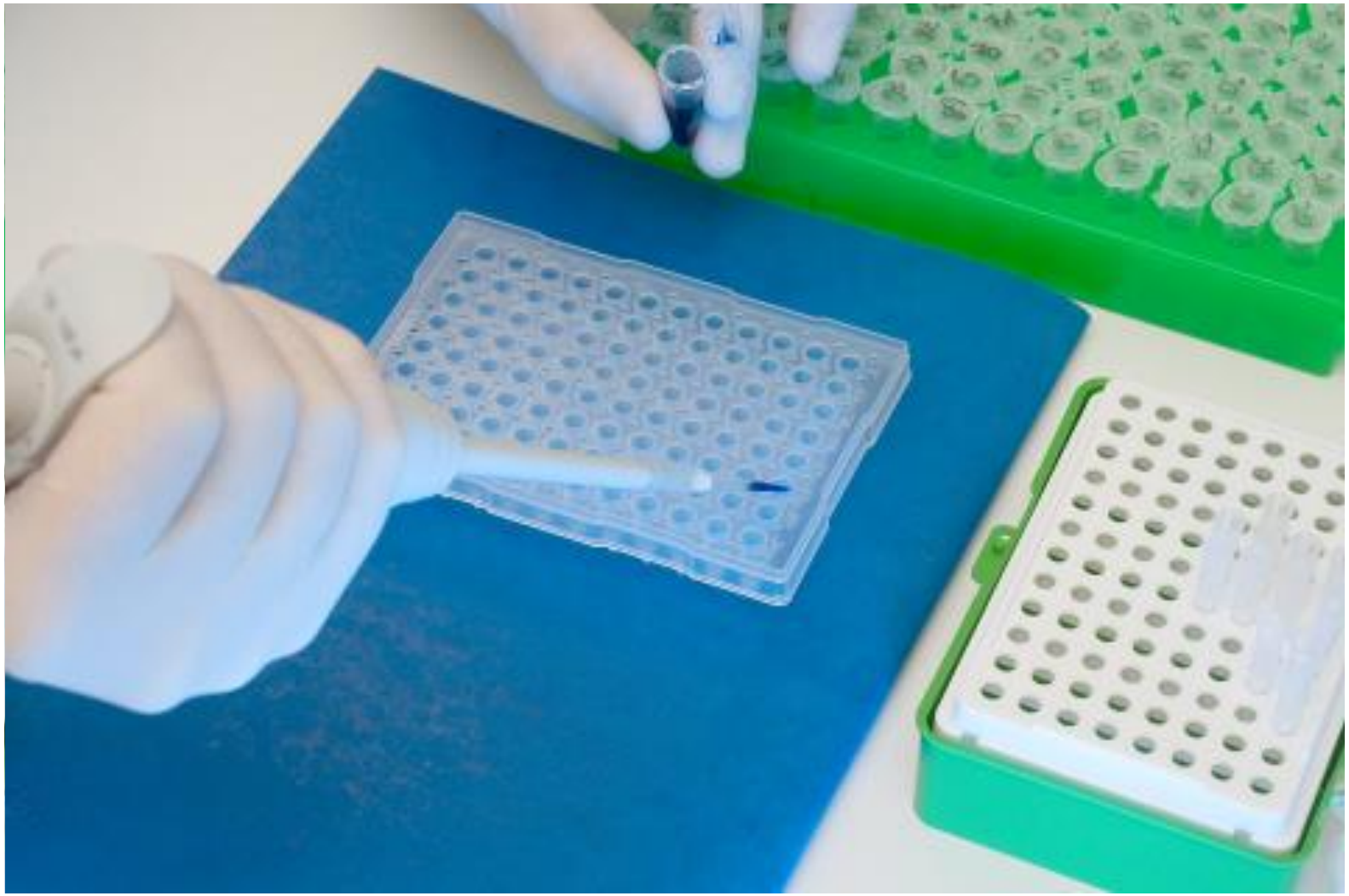
Identifizierung und Quantifizierung
des Tumorzellgehalts auf dem Objektträger



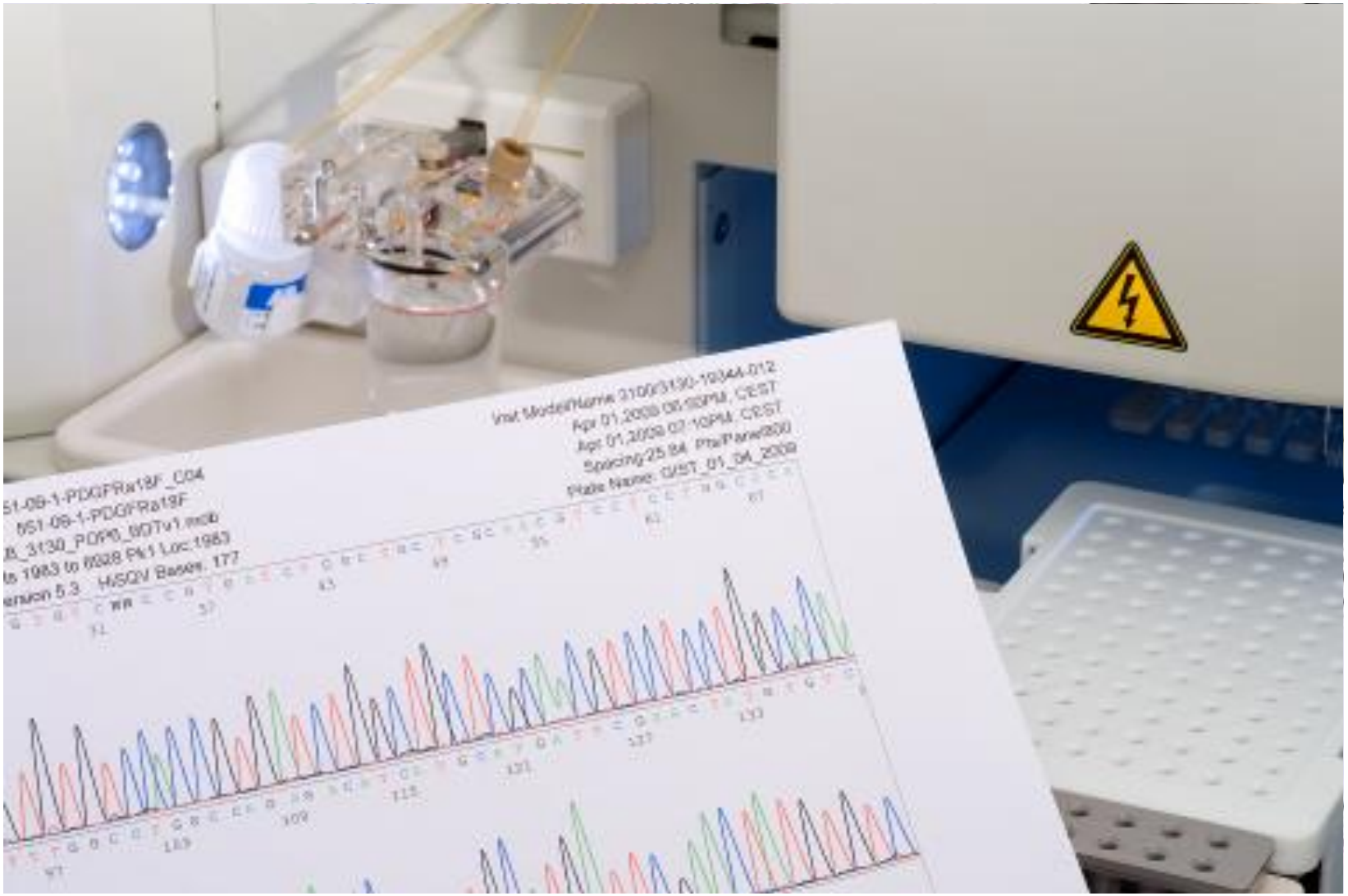
Makrodissektion

Entparaffinierung

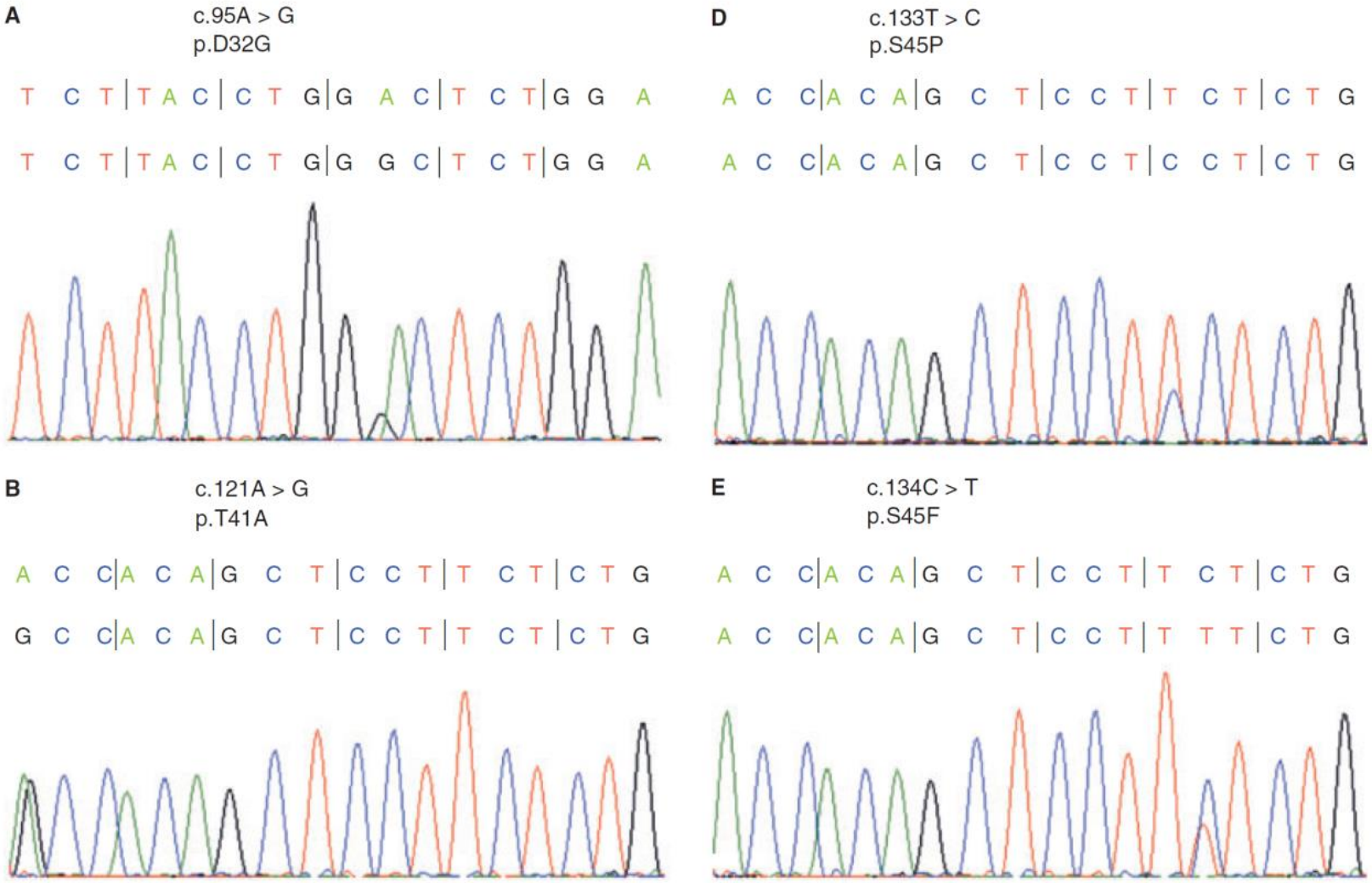
Mutationsanalyse



Sanger-Sequenzierung – jahrelanger Goldstandard

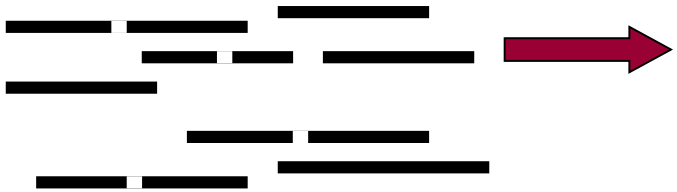


Sanger-Sequenzierung

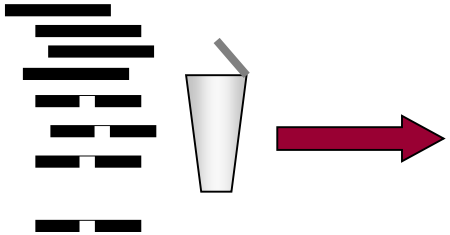


Next generation sequencing: NGS

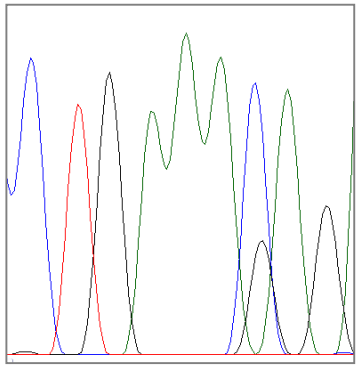
1st Generation Sequencing



DNA population



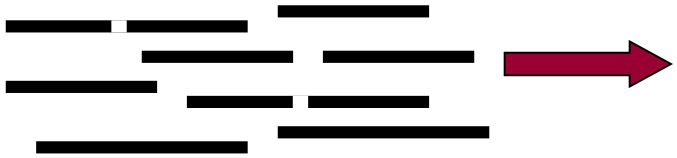
Simultaneous amplification

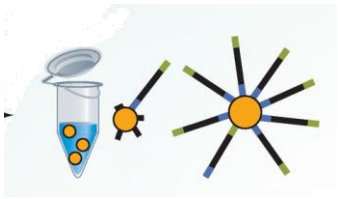


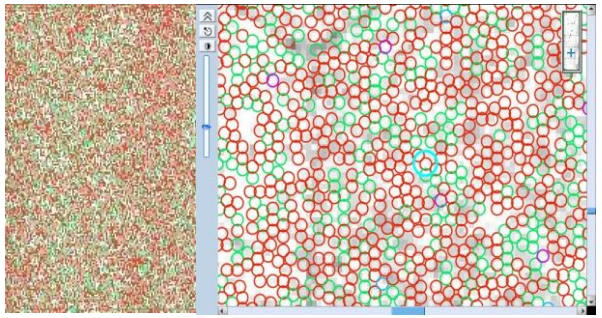
Simultaneous sequencing of the amplicon population

Next Generation Sequencing

DNA population







Adaptor ligation (Library)

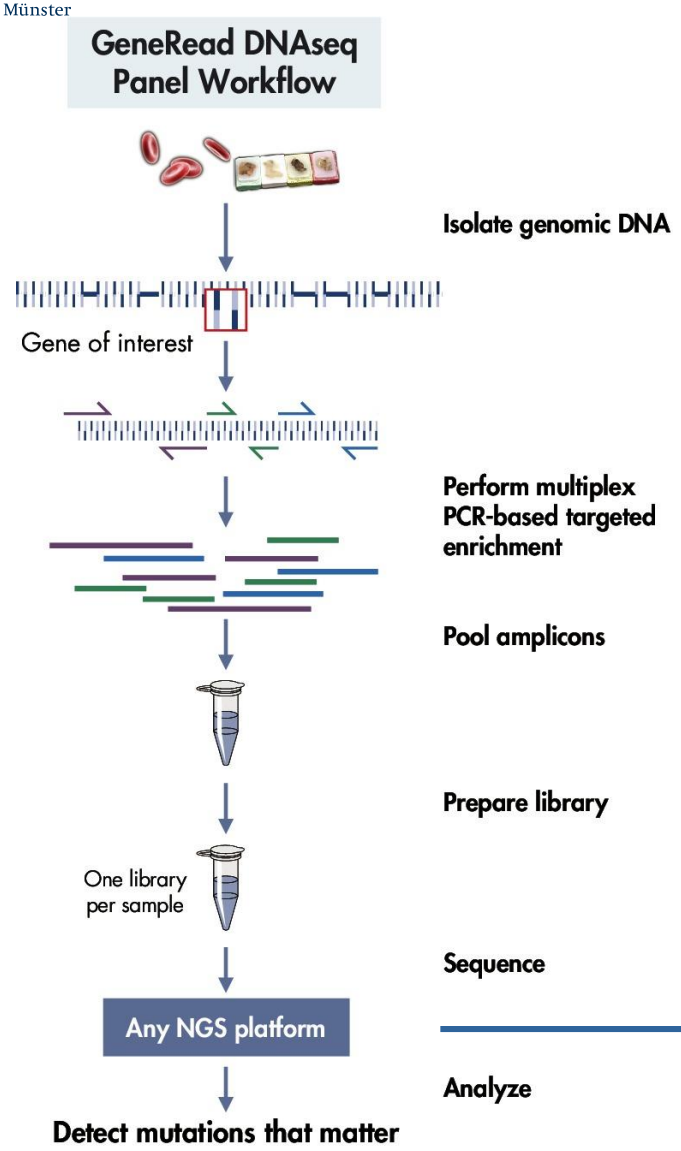
clonal amplification of single molecules

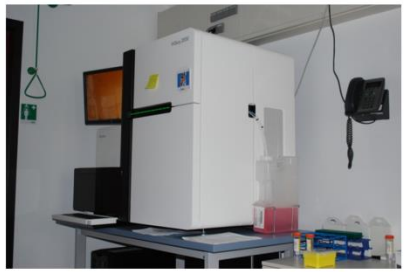
Parallel sequencing of the amplicon clones

Vorteile des next generation sequencing

- wenig DNA Gehalt erforderlich
- simultane Analyse vieler Gene gleichzeitig
- Menge der mutierten DNA kann quantifiziert werden
- kleine Mengen von Subklonen können nachgewiesen werden

Next Generation Sequencing - Workflow

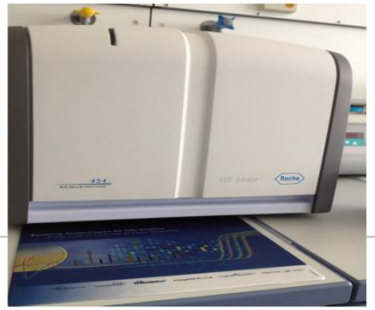




Illumina HiSeq + MiSeq

Ion Torrent PGM

Roche GS FLX



Roche 454 Junior

18

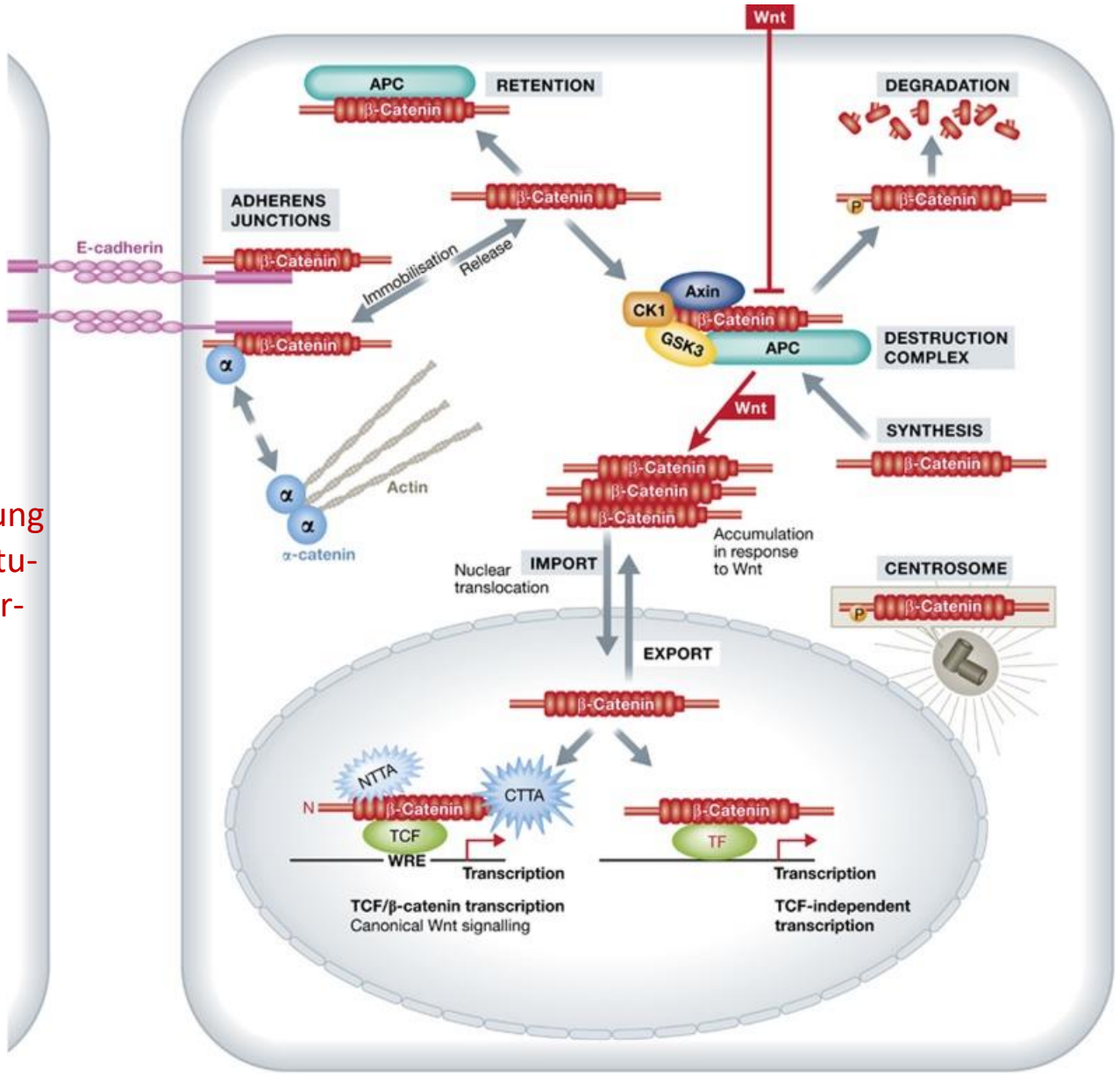
Seite 18

MiSeq - Illumina

ß-Catenin in der Zelle

Funktionen:

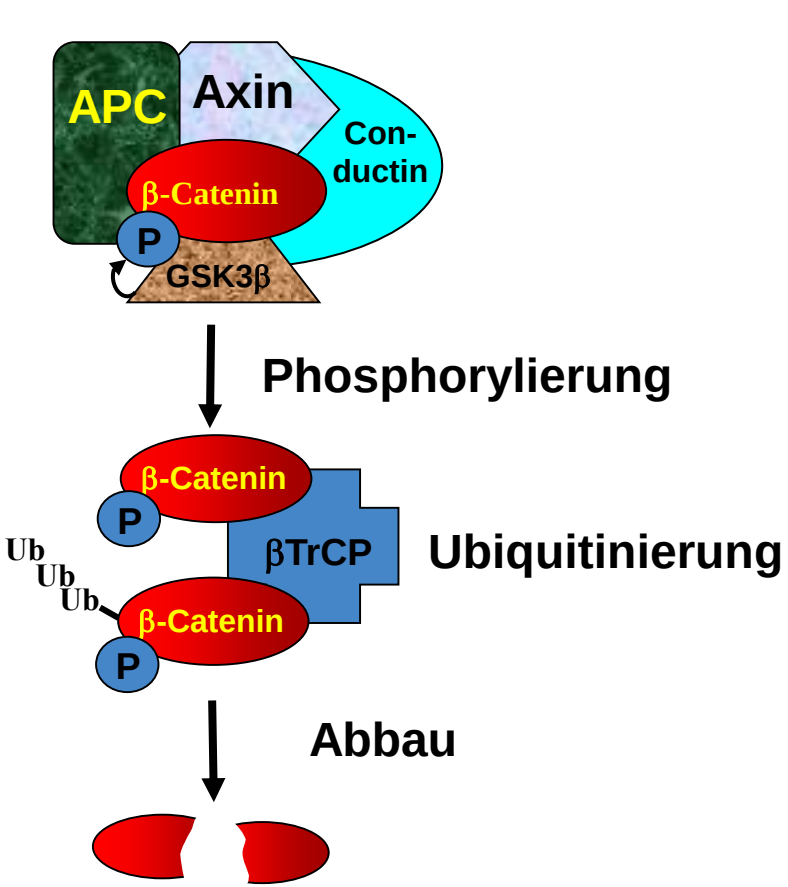
- Duale Rolle in der Entwicklung Signalweitergabe und strukturelle Entwicklung der Körperachse
- Gewebshomeostase
- Zellerneuerung
- Regeneration
- …



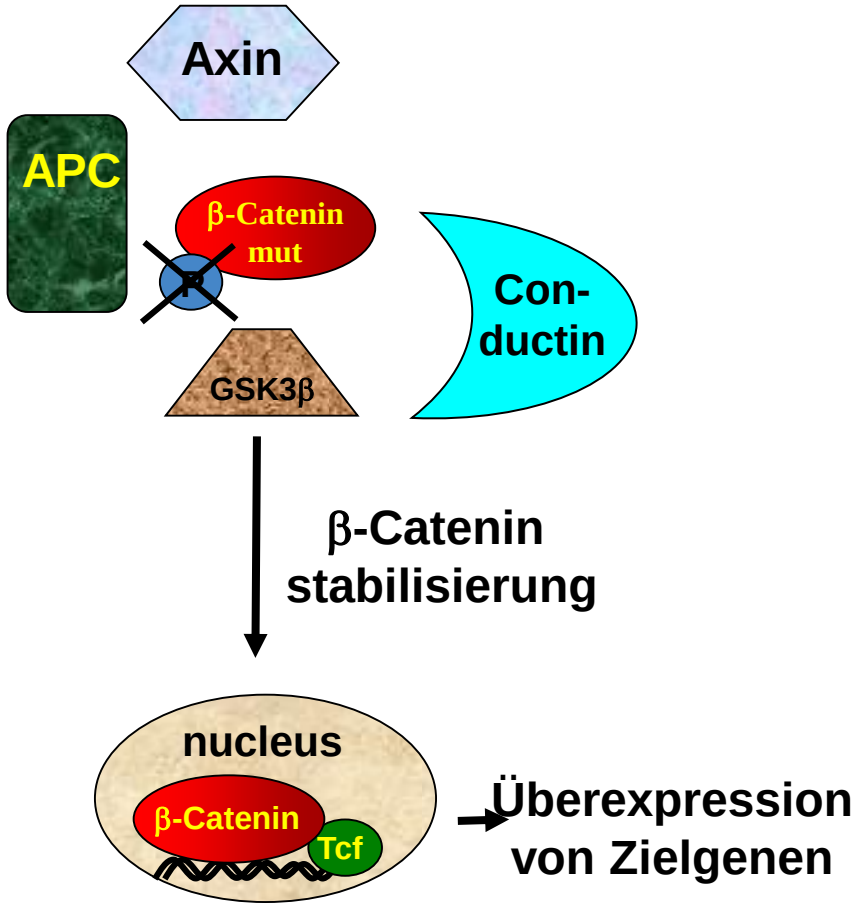
Valenta et al. EMBO J 2012

Der ß-Cateninspiegel und die Lokalisation in der Zelle sind variabel

Physiologischer Abbau



Mutationsbedingte Hemmung des Abbaus



Courtesy of Arend Koch

Posttranslationale Regulierung von ß-Catenin

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

MODIFICATION	SITES	ENZYME	FUNCTION (enhancement of)	REFERENCES
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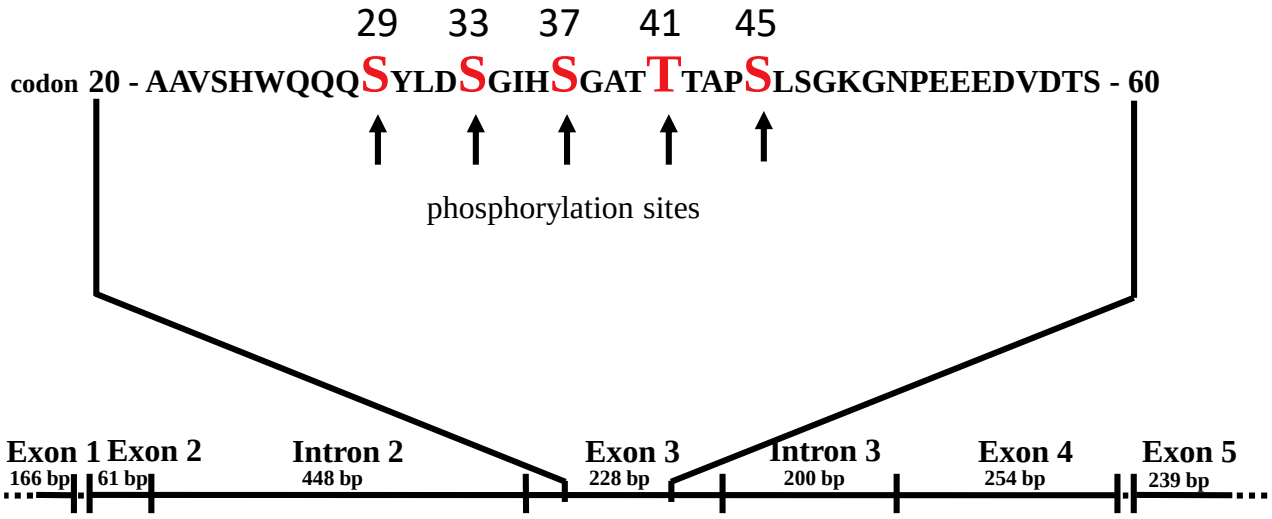
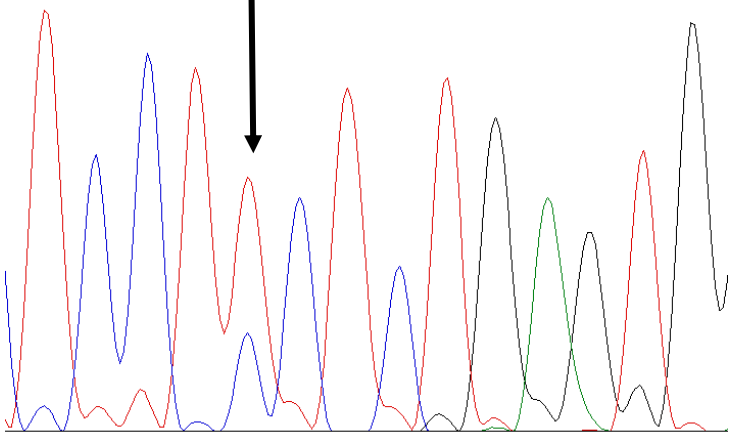
Valenta et al. EMBO J 2012

β-Cateninphosphorylierungsstellen



T C T Wild type
C C T mutant sequence



Point mutation in codon 45 (Ser-45Pro)

Courtesy of Arend Koch

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

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

Häufige Mutationstypen bei Fibromatose

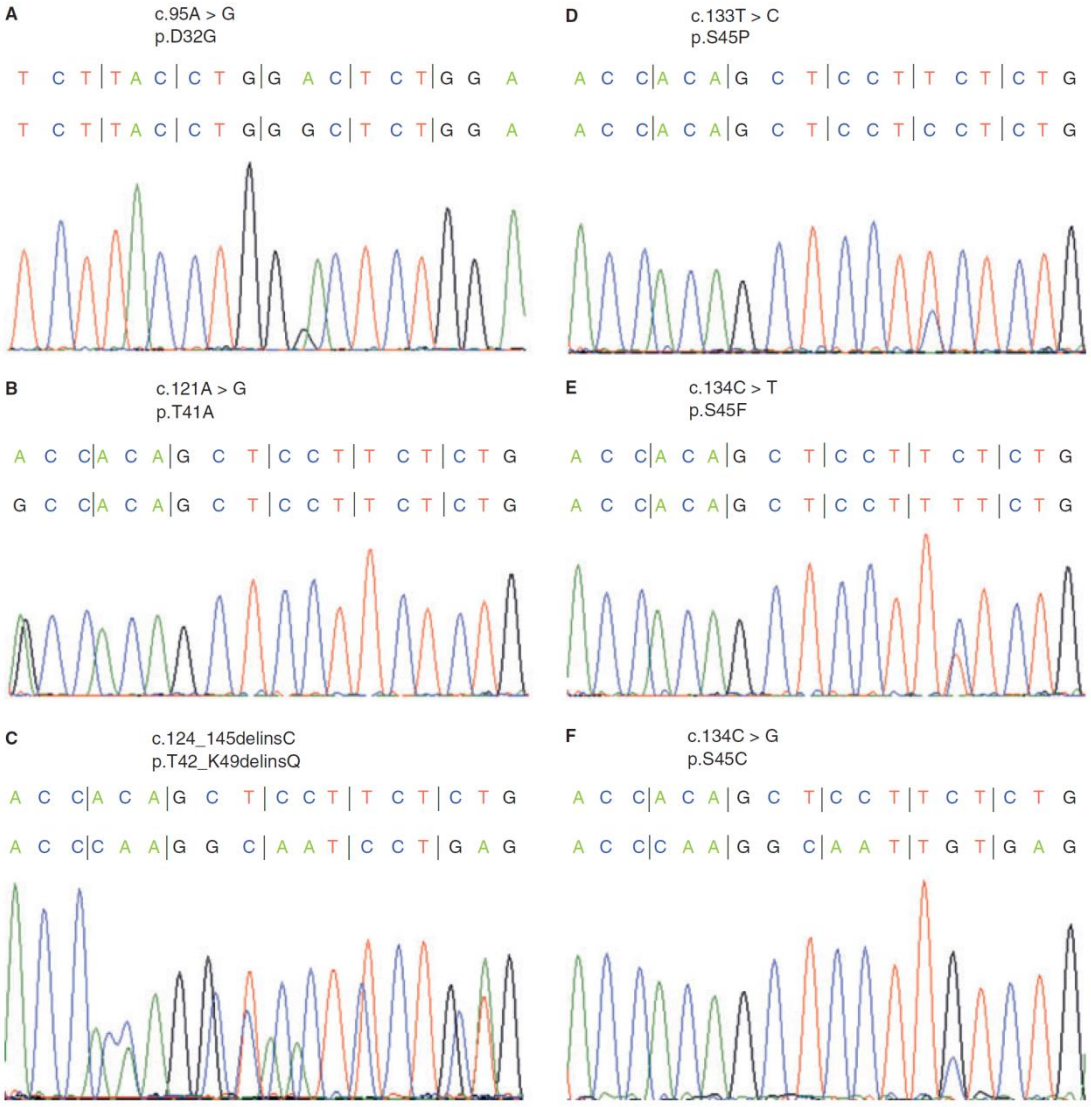


Figure 1. *CTNNB1* mutations in our cohort. β-Catenin mutations were detected in 84% of desmoid fibromatoses. Heterozygous point mutations included codon 32 (**A**), codon 41 (**B**) and codon 45 (**D–F**). (**C**) shows a deletion/insertion mutation involving codon 45.

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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

Korrelation der ß-Cateninexpression und *CTNNB1*-Mutation

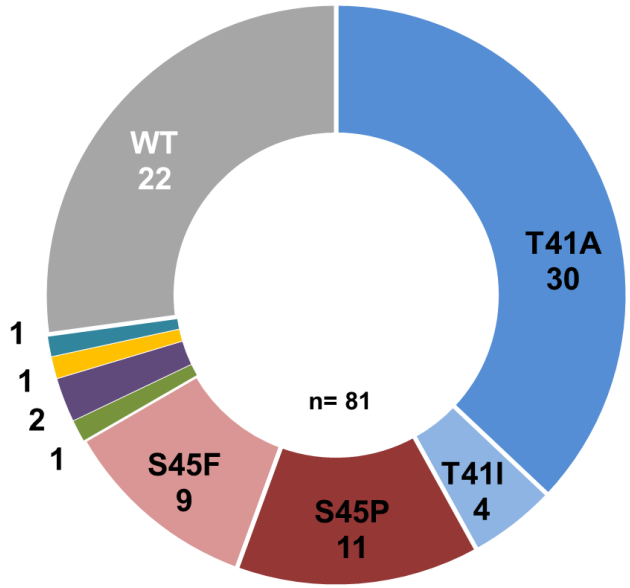
CTNNB1 Mutation		Nucleäre ß-Cateninexpression	
		Positiv	**Negativ**
D32G		1	0
T41A		54	4
S45F		5	0
S45P		4	1
S45C		1	0
T42_K49delinsQ		1	0
Wild type	Associated with FAP	3	2
	Not associated with FAP	8	0
Total		77	7

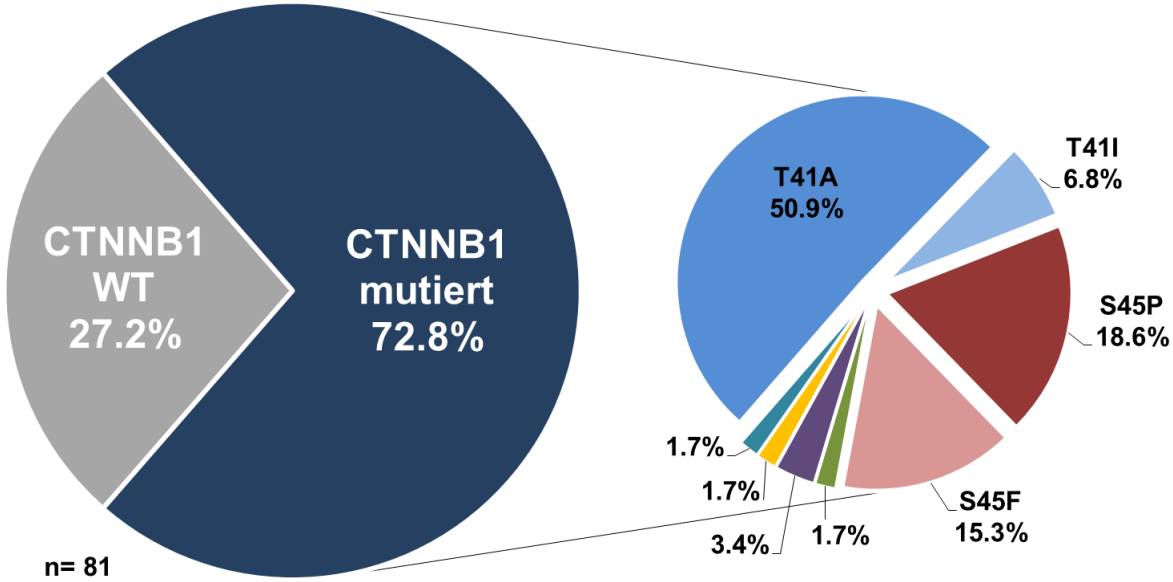
Huss, S. et al. Histopathology 2013

Mutationstypen in CTNNB1 bei Fibromatose (neue Kohorte seit 2014)

	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) *double mutant	85	100

CTNNB1 (Exon 3) Mutationen





c.[133T>A(;)134C>A]; p.[(S45T(;)S45Y)]
c.[133T>C(;)134C>T]; p.[(S45P(;)S45F)]
c.[134C>T(;)135T>C]; p.[(S45F(;)S45S)]
c.133delinsAAGG; p.S45delinsKA

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

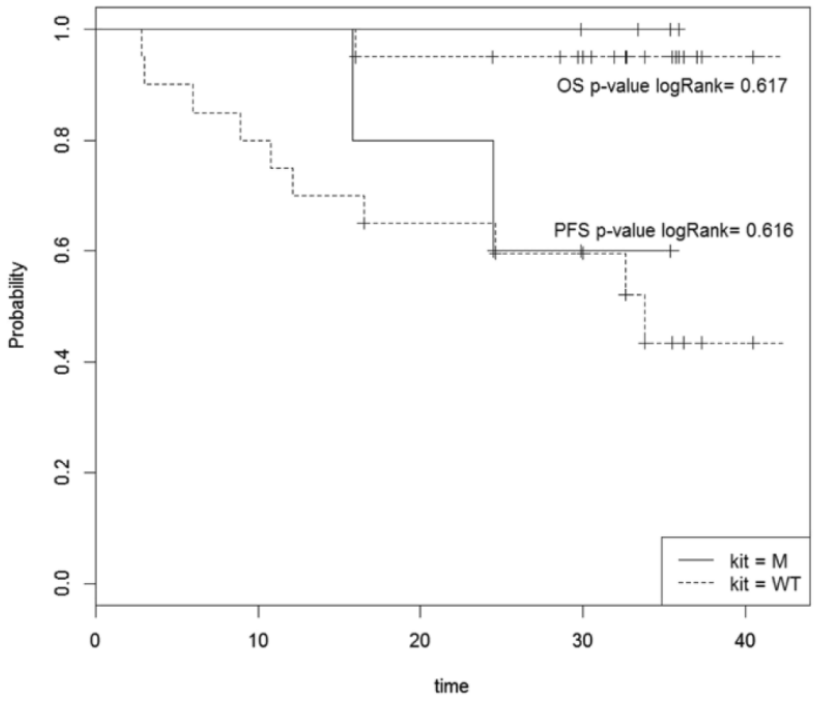


Figure 4 Log-rank analysis of progression-free survival (PFS) and overall survival (OS) for patients with (M) and without (WT) KIT^{L541} variant in phase II Desminib trial.

Prognostische Relevanz des Mutationstyps in *CTNNB1* bei Fibromatose

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

Summary

- Die Analyse des Mutationsstatus in *CTNNB1* (Exon 3) ist einfach
- S45F könnte ein Indikator für aggressivere Typen sein
- S45 Mutationen sprechen besser auf Imatinib an als T41 Mutationen und Wildtypfälle
- Einige Fibromatosen sind möglicherweise polyklonal
- Die Therapiestrategien sind vielfältig, zunehmend wird auch abgewartet („watch and wait“)
- Next generation sequencing ist ein wertvolles Werkzeug, um die Entstehung und Biologie von Fibromatosen besser zu verstehen

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Review

The management of desmoid tumours: A joint global consensus-based guideline approach for adult and paediatric patients

The Desmoid Tumor Working Group[1]

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KEYWORDS
Desmoid tumour;
β-catenin;
CTNNB1;
Gardner syndrome;
Medical therapy;
Radiotherapy;
Surgery;
Patient advocacy groups;
SPAEN;

Abstract Desmoid tumor (DT; other synonymously used terms: Desmoid-type fibromatosis, aggressive fibromatosis) is a rare and locally aggressive monoclonal, fibroblastic proliferation characterised by a variable and often unpredictable clinical course. Previously surgery was the standard primary treatment modality; however, in recent years a paradigm shift towards a more conservative management has been introduced and an effort to harmonise the strategy amongst clinicians has been made. We present herein an evidence-based, joint global consensus guideline approach to the management of this disease focussing on: molecular genetics, indications for an active treatment, and available systemic therapeutic options. This paper follows a one-day consensus meeting held in Milan, Italy, in June 2018 under the auspices of the European Reference Network for rare solid adult cancers, EURACAN, the European Organisation for Research and Treatment of Cancer (EORTC) Soft Tissue and Bone Sarcoma Group (STBSG) as well as Sarcoma Patients EuroNet (SPAEN) and The Desmoid tumour Research Foundation (DTRF). The meeting brought

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