

Mutationsanalyse und andere genetische Untersuchungen bei GIST – Was ist sinnvoll?

Was macht der Pathologe/die Pathologin?



Die Basis: Das Lichtmikroskop!



Mikroskopieren – allein, zu zweit, mit vielen....



Färbeautomat für die Immunhistochemie



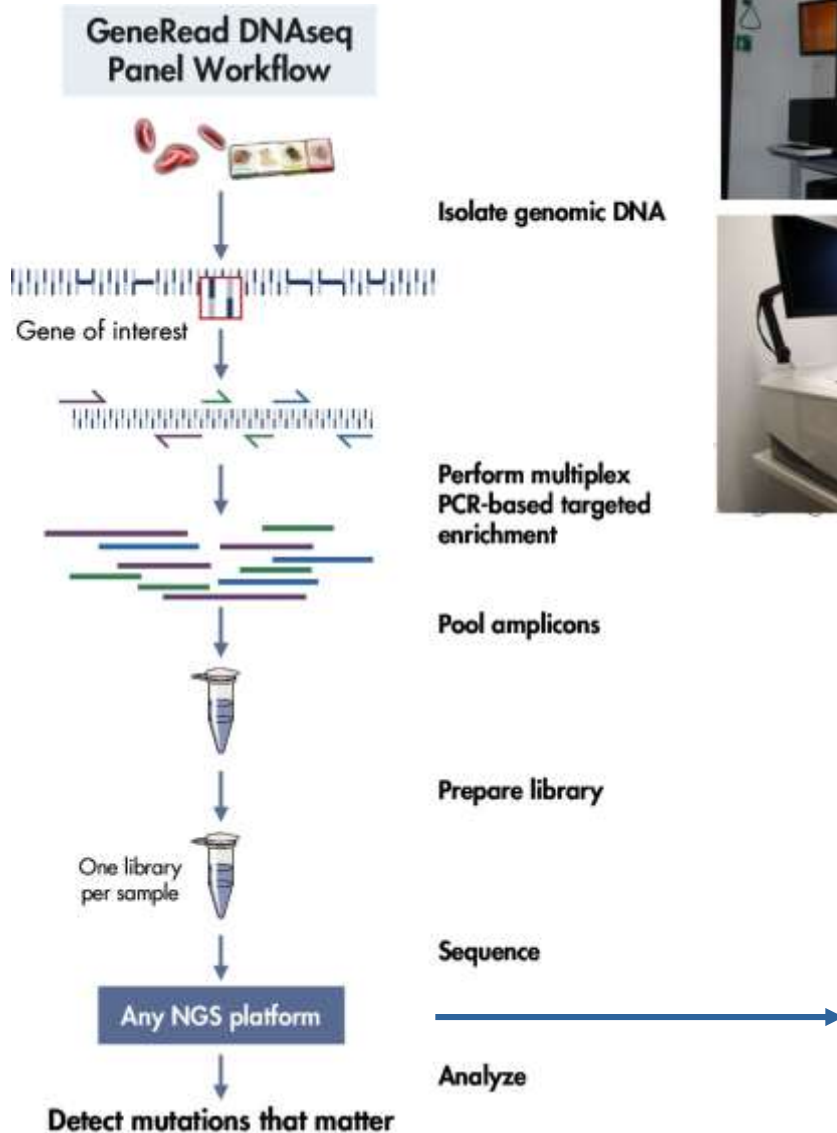
Fluoreszenz-in situ-Hybridisierung = FISH



Sequenzierung der DNA/RNA von Tumoren



Next Generation Sequencing (NGS) - Workflow



Illumina
HiSeq +
MiSeq



Ion Torrent
PGM



Roche
GS FLX



Roche
454 Junior

MiSeq - Illumina



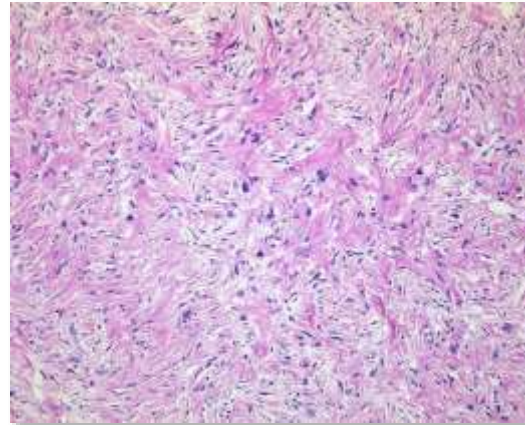
Vorteile des Next Generation Sequencing (NGS)

- geringe DNA Menge reicht aus
- simultane Analyse zahlreicher Gene
- Quantifizierung der mutierten DNA möglich
- Detektion minorer (resistenter) Subklone möglich

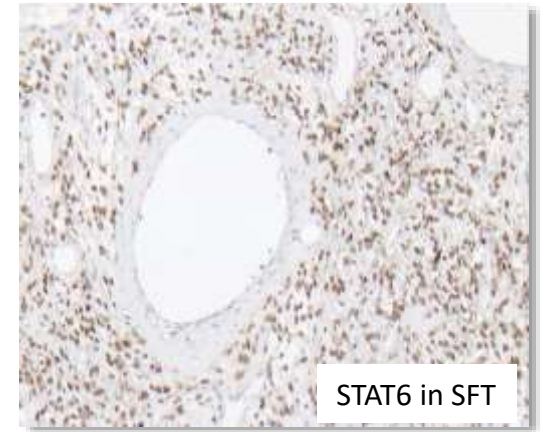
Welche diagnostischen Werkzeuge haben Pathologen?



Makroskopie



Mikroskopie

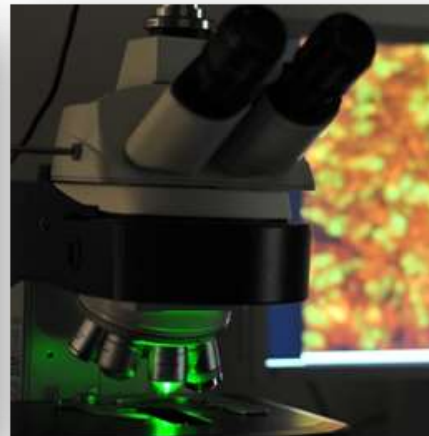


Immunhistochemie

Molekularpathologie



Sanger-Sequenzierung



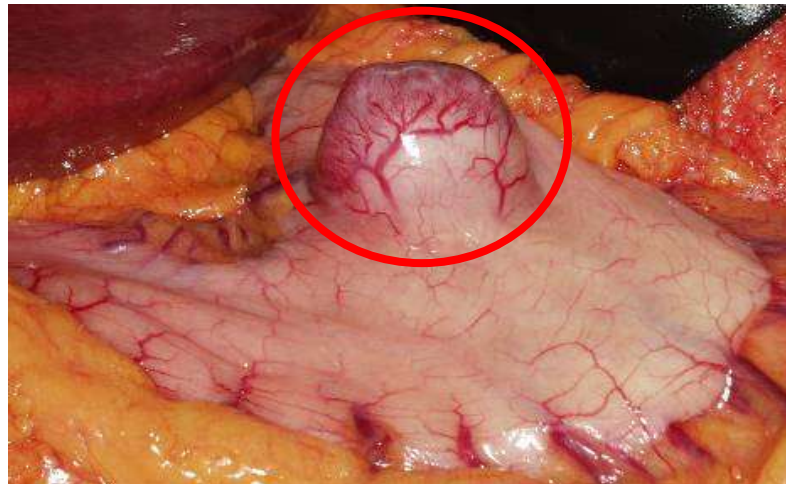
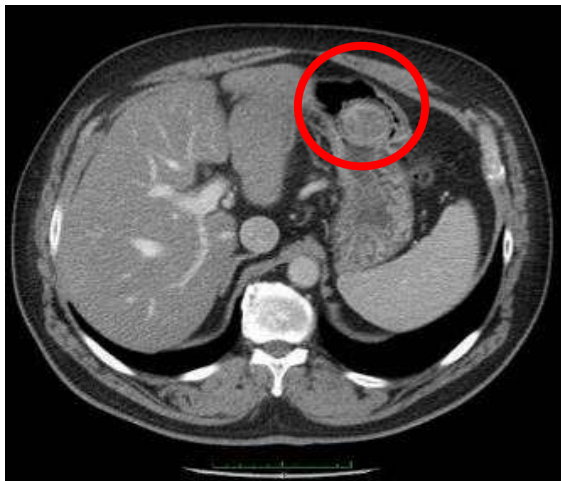
FISH



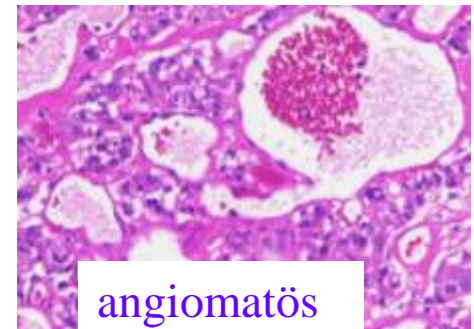
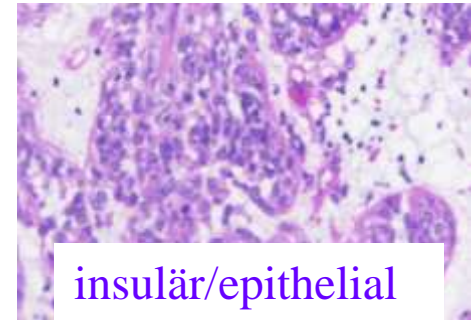
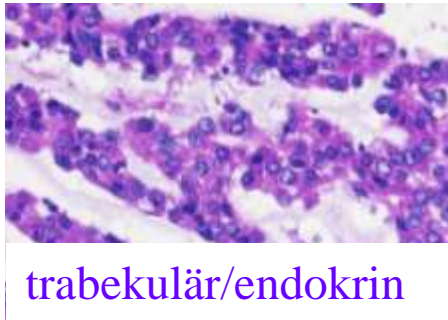
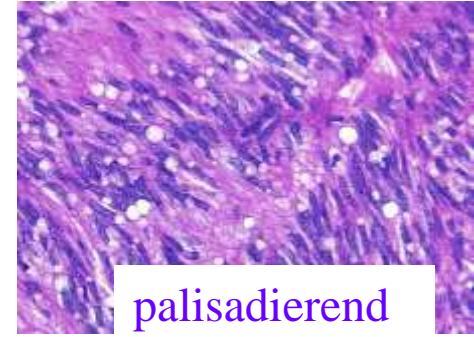
Next Generation Sequencing (NGS)

Gastrointestinale Stromatumoren (GISTs)

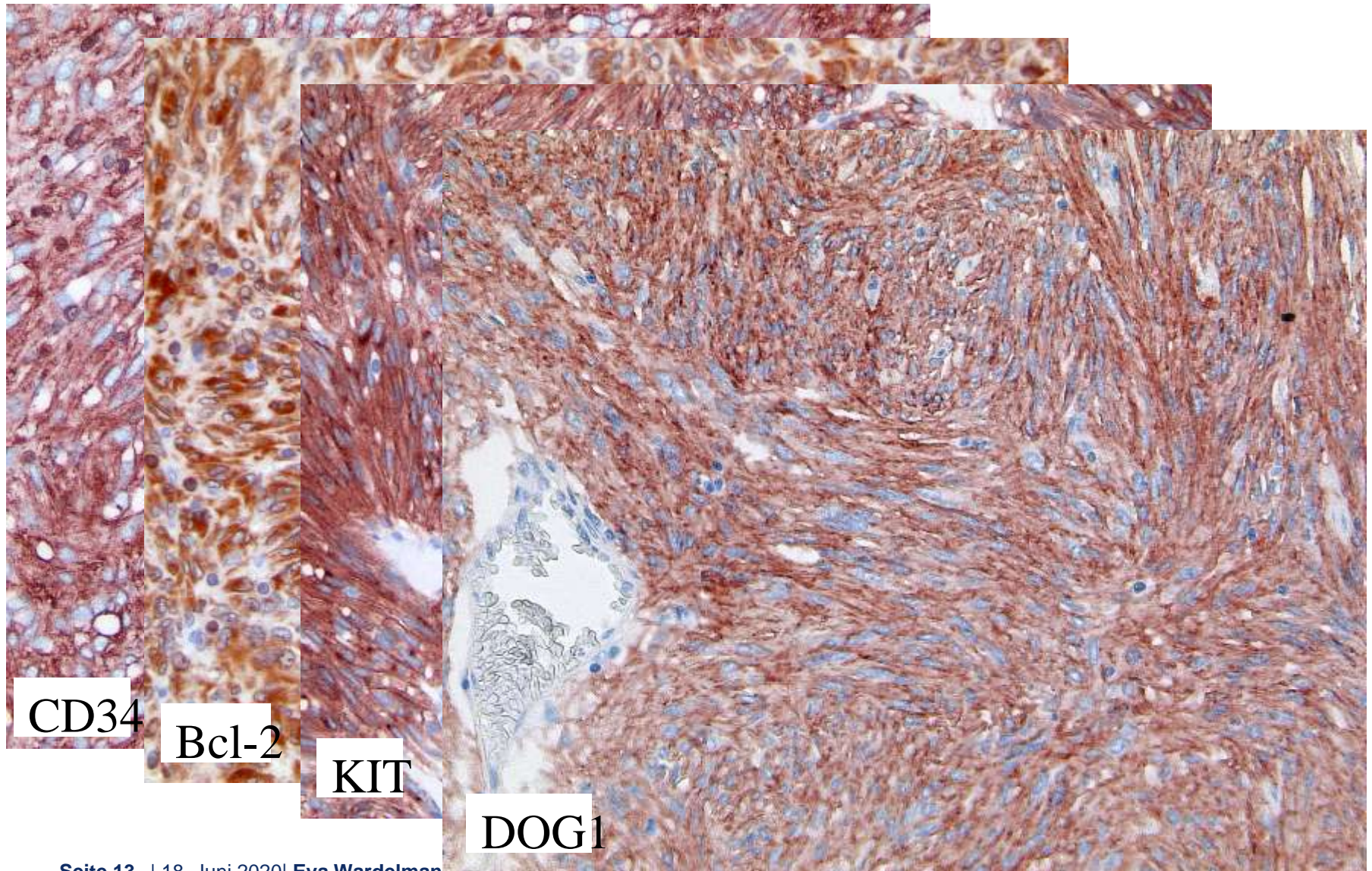
- häufigste mesenchymale Tumoren im Gastrointestinaltrakt
- Inzidenz 10-20/Mio. und Jahr
- ca. 1200 Neuerkrankungen/Jahr in Deutschland
- Altersgipfel zw. 60 und 70 Jahre
- ~ 50% aller GIST metastasieren



Histologisches Erscheinungsbild von gastro-intestinalen Stromatumoren



Immunhistochemie bei GIST

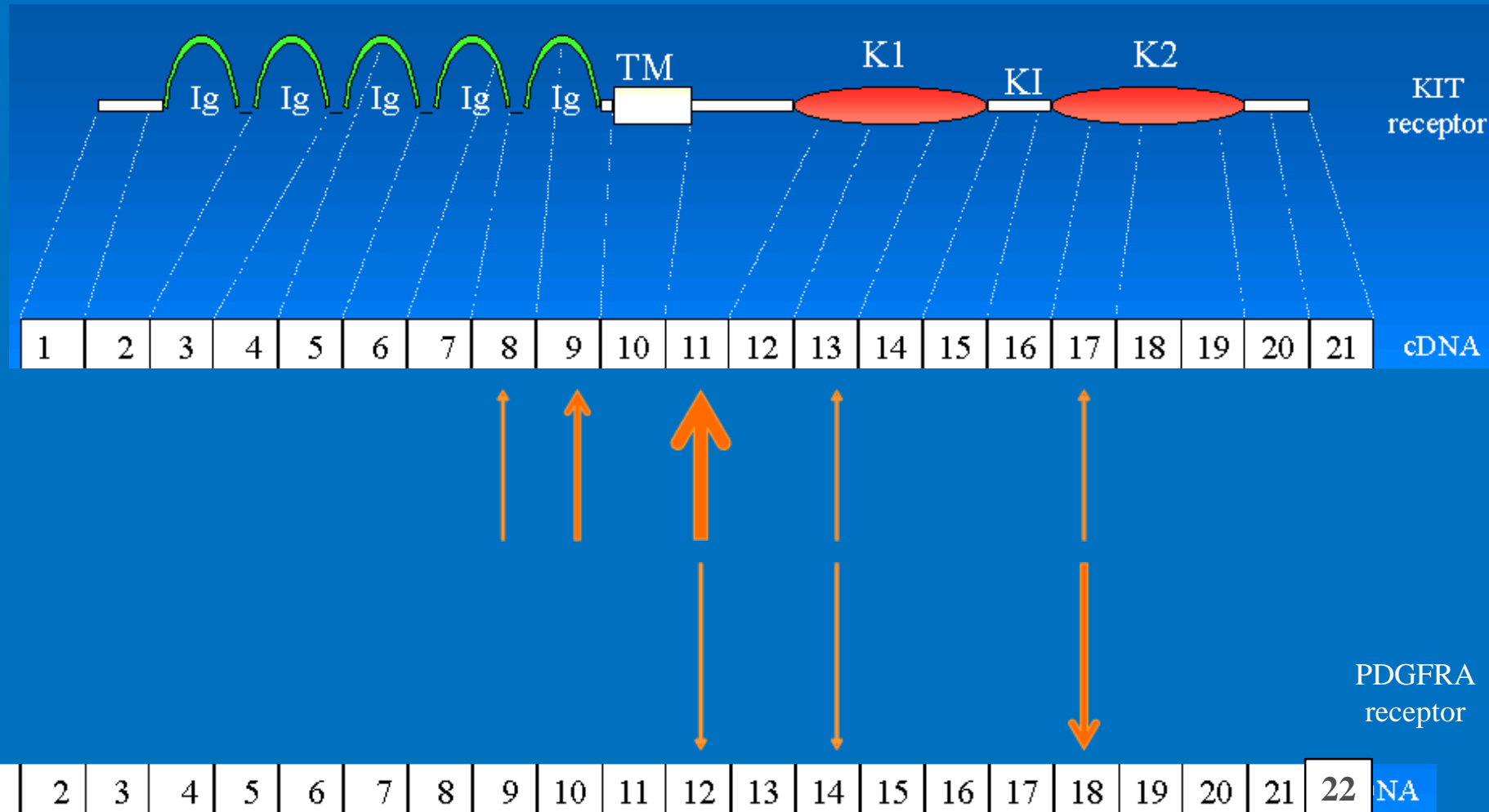


Risikoklassifikation (NCCN-AFIP criteria)

| group | size | Mitotic count | Risk | | | |
|-------|-----------|----------------------|---------------------|---------------------|-----------------|-----------------|
| | | | stomach | jej./ileum | duodenum | rectum |
| 1 | ≤ 2 cm | ≤5/5 mm ² | ∅ | ∅ | ∅ | ∅ |
| 2 | > 2-5 cm | ≤5/5 mm ² | very low (1,9%) | low (4,3%) | low (8,3%) | low (8,5%) |
| 3a | > 5-10 cm | ≤5/5 mm ² | low (3,6%) | moderate (24,0%) | high (34,0%) | high (57,0%) |
| 3b | > 10 cm | ≤5/5 mm ² | moderate (12,0%) | high (52,0%) | high (34,0%) | high (57,0%) |
| ----- | | | | | | |
| 4 | ≤ 2 cm | >5/5 mm ² | ∅* | high* (50,0%) | - | high (54,0%) |
| 5 | > 2-5 cm | >5/5 mm ² | moderate (16,0%) | high (73,0%) | high (50,0%) | high (52,0%) |
| 6a | > 5-10 cm | >5/5 mm ² | high (55,0%) | high (85,0%) | high (86,0%) | high (71,0%) |
| 6b | > 10 cm | >5/5 mm ² | high (86,0%) | high (90,0%) | high (86,0%) | high (71,0%) |

* Low case numbers

Schwerpunktregionen von *KIT*- und *PDGFRA*-Mutationen

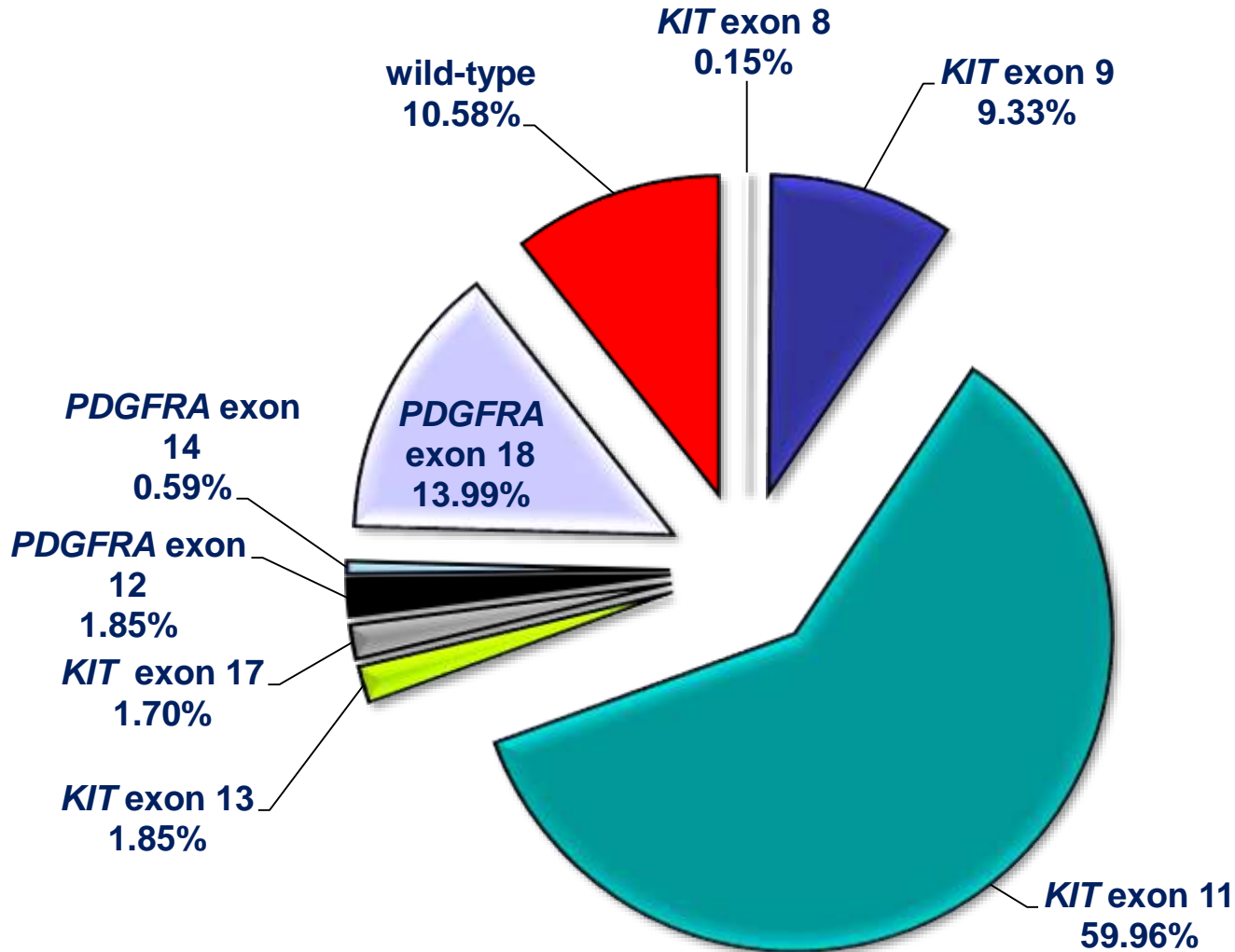


KIT-Gen: 21 Exons

PDGFRA-Gen: 22 Exons

beide lokalisiert auf Chromosom 4q12

KIT und *PDGFRA* Mutationen treten in 85% bis 90% der GIST auf (n=1351)



Der Mutationstyp ist relevant für die Prognose und das Therapieansprechen (Prädiktion) auf Imatinib bei GIST

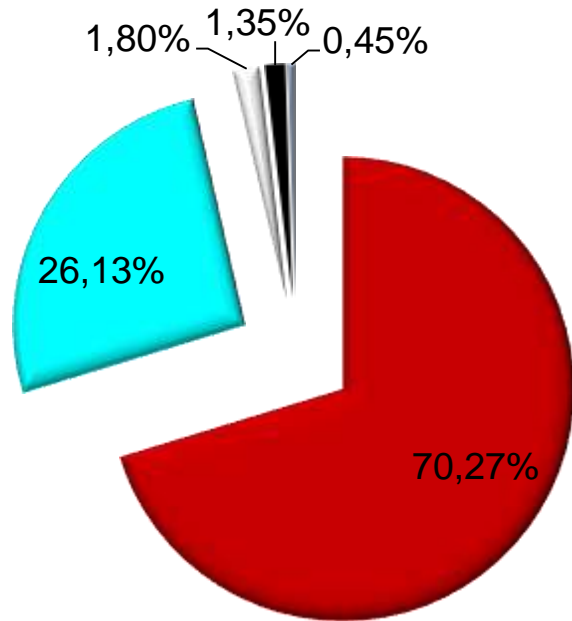
Prognose

- aggressivere Subtypes (z. B. Deletionen in *KIT* Exon 11)
- weniger aggressive Subtypen (*PDGFRA* Mutationen)

Prädiktion

- *KIT* Exon 11 bestes Ansprechen
- *KIT* Exon 9 mittleres Ansprechen
- *PDGFRA* Exon 18 D842V resistent
- sekundäre *KIT* Mutationen als Ursache eines Therapieversagens

Häufigkeit der Resistenzmutation p.D842V



- p.D842V (n=156)
- PDGFRA exon 18 deletions (n=58)
- p.D842I (n=4)
- p.D842Y (n=3)
- p.Y849C (n=1)

- **15% aller GIST tragen eine *PDGFRA* Exon 18 Mutation**
- **nahezu alle sind im Magen lokalisiert**

~70% aller in Magen-GIST lokalisierten *PDGFRA* Exon 18 Mutationen sind p.D842V!

Insgesamt tragen 10% aller GIST die Resistenzmutation p.D842V!

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

Sebastian Huss¹, Helen Künstlinger¹, Eva Wardelmann¹, Michaela A Kleine¹, Elke Binot¹, Sabine Merkelbach-Bruse¹, Thomas Rüdiger², Jens Mittler³, Wolfgang Hartmann¹, Reinhard Büttner¹ and Hans-Ulrich Schildhaus¹

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) | <i>KIT</i> 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) | <i>KIT</i> 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.

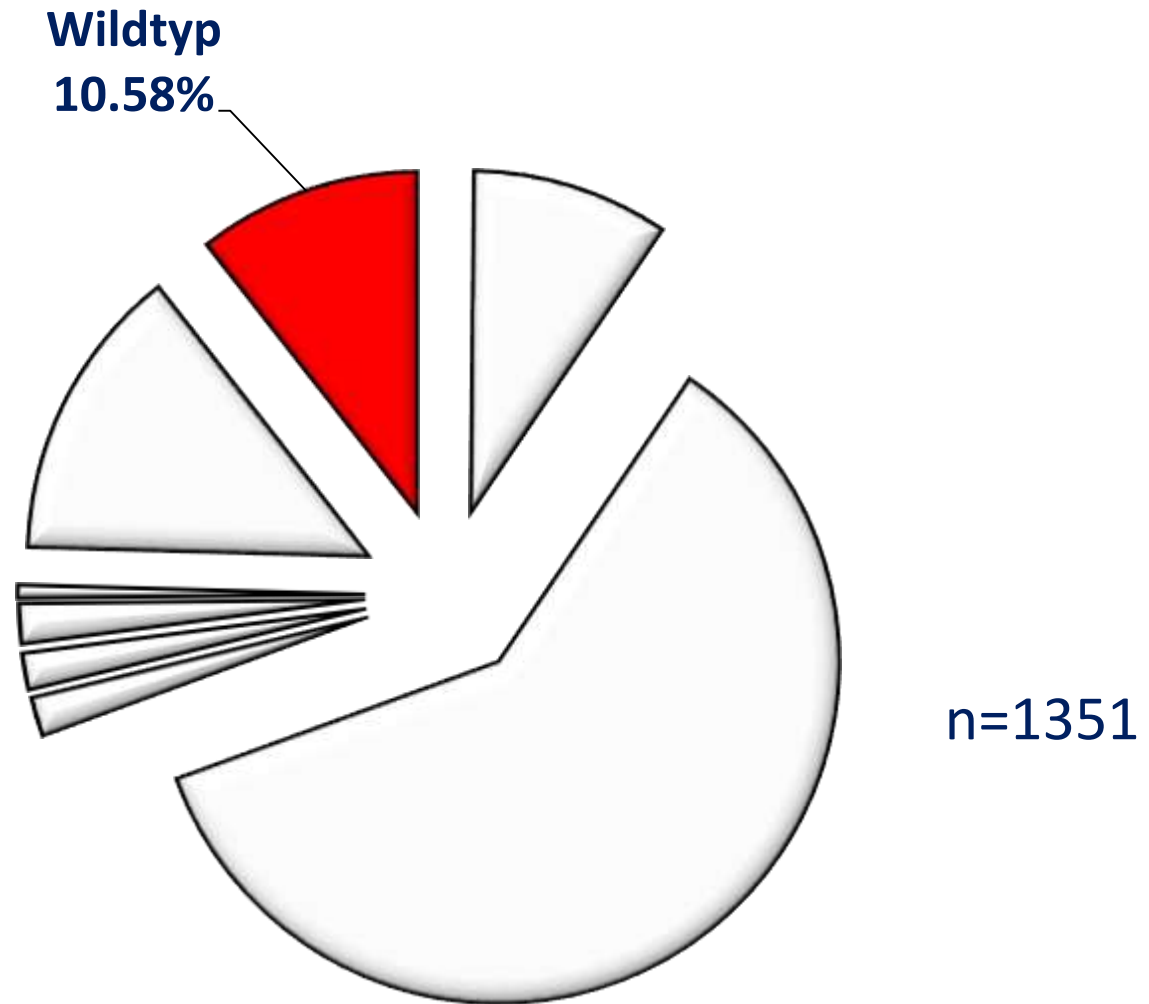
^aAccording to the NCCN-AFIP criteria.²⁸

2 Fälle unter 145 wt GIST (1.38% der wt GIST, 0.15% aller GIST)

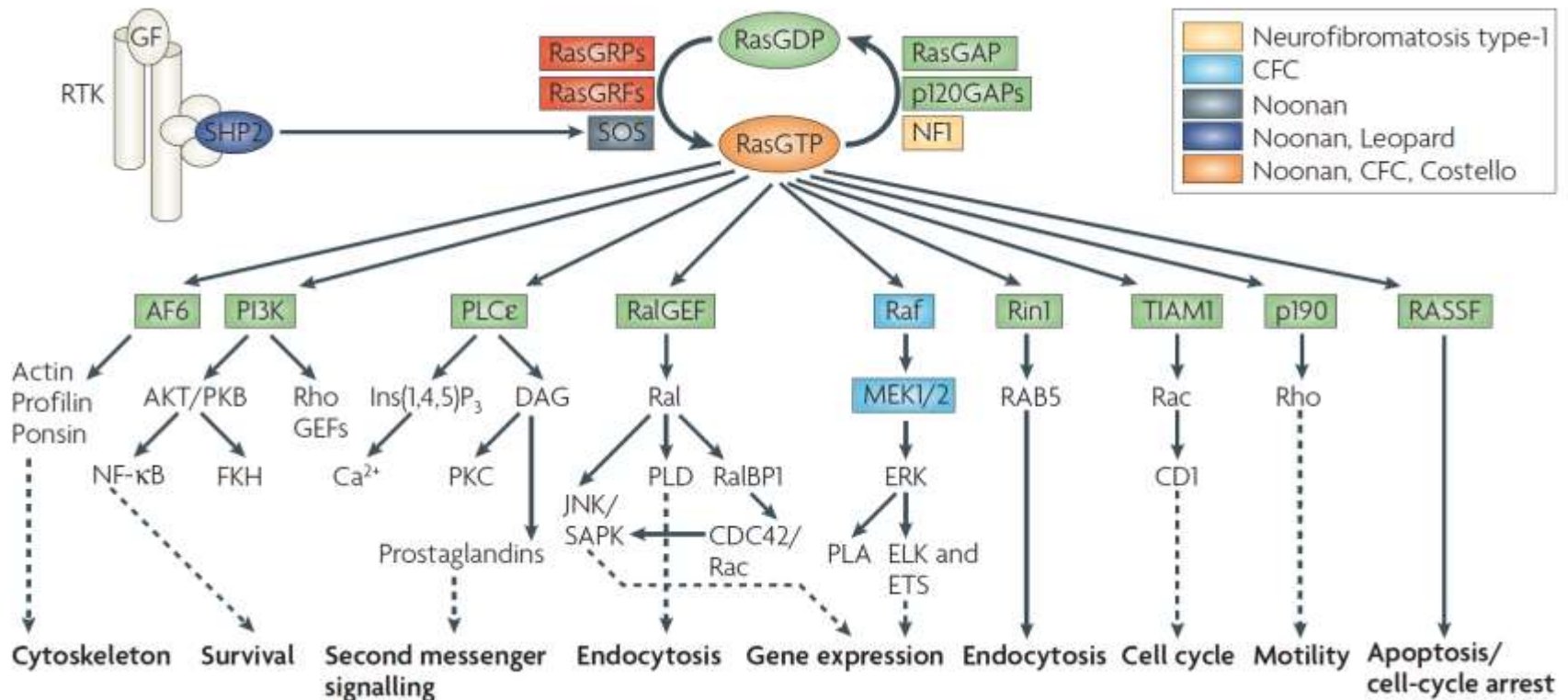
Ito et al. IJEP 2014: 3 Fälle unter ~ 1000 GIST (0.3%)

Lokalisation: Dünndarm
Biologie: können aggressiv sein

Und jenseits von *KIT* und *PDGFRA* Mutationen?



Alternative Mutationen bei GIST ohne KIT/PDGFR α Mutation - Neurofibromatose Typ 1 Mutationen -



Karnoub and Weinberg, Nature reviews 2008

Die Häufigkeit von GIST bei NF1 Patienten ist 7%.

KRAS Mutationen bei GIST ohne KIT und PDGFRA Mutation

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 Nafa¹

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 |

GCC, 2014

Alternative Mutationen bei GIST ohne *KIT/PDGFR*A Mutation - *BRAF*-Mutationen -

Agaram GCC 2008

BRAF MUTATIONS IN GIST

855

TABLE I. 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 | PI6 | 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.

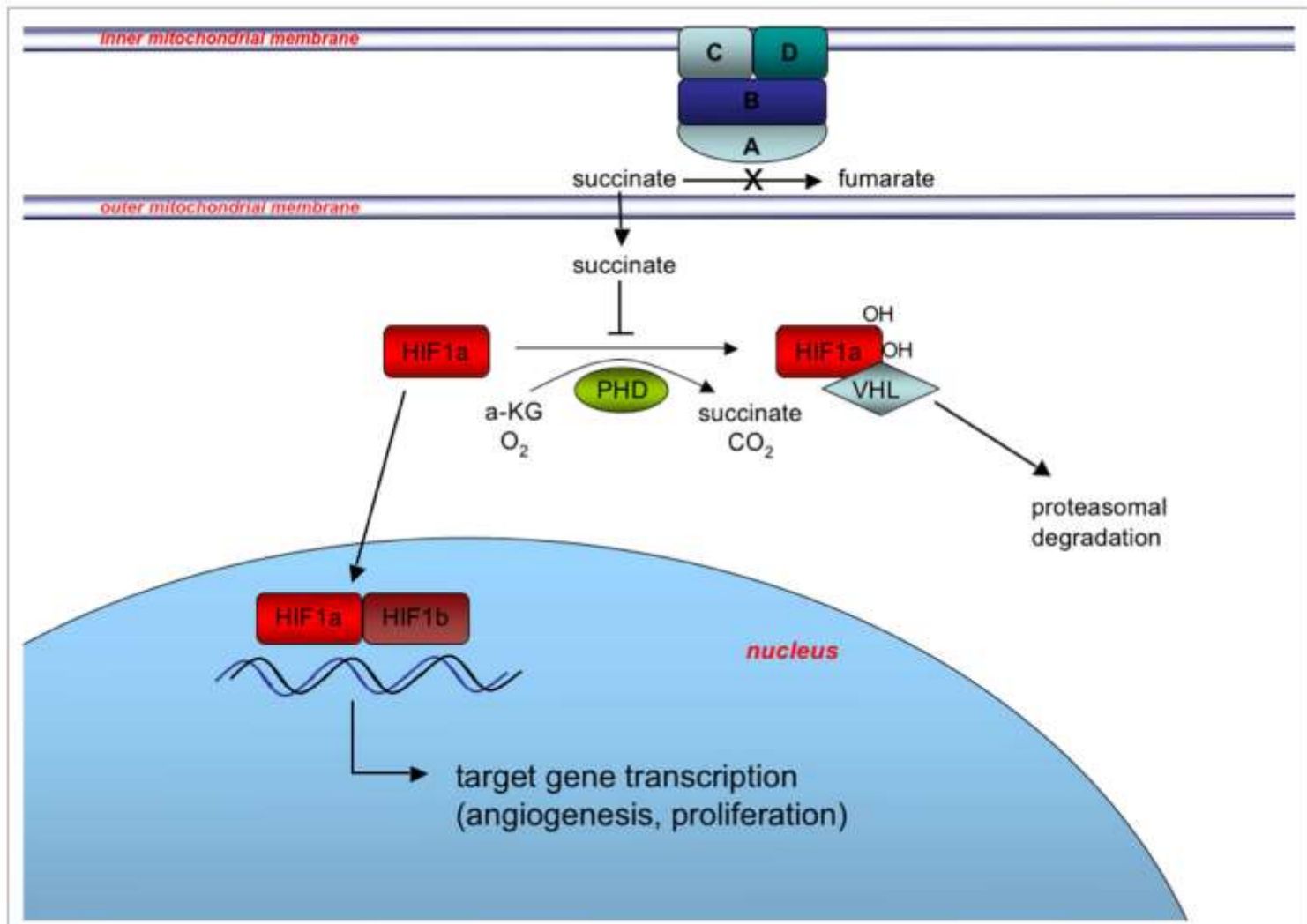
^aGenotyping on normal tissue as well.

^bImatinib resistant GIST.

| | | wt | <i>KIT</i> -mutant | <i>PDGFR</i> A-mutant |
|-------------|-----------|----------|--------------------|-----------------------|
| BRAF | wild type | 172 | 228 | 44 |
| | mutant | 7 (3.9%) | 0 | 0 |
| | | 179 | 228 | 44 |

Huss, S et al. Hum Pathol 2017

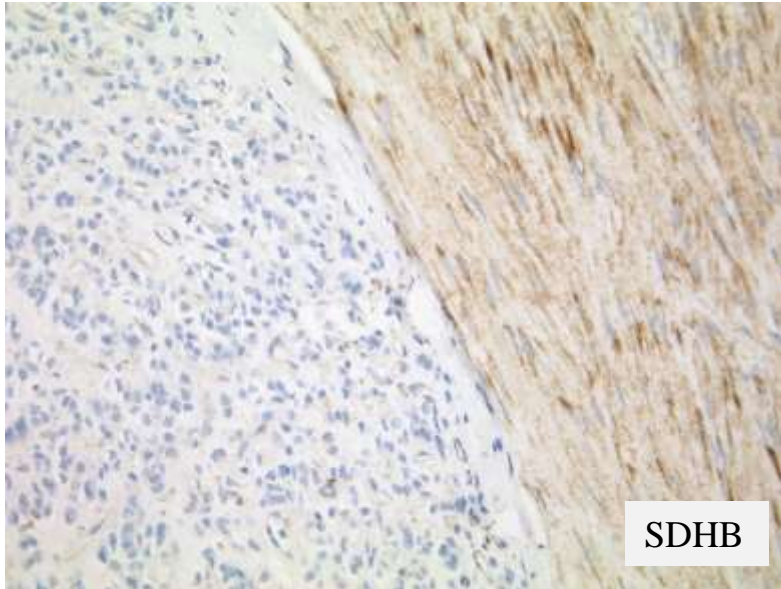
Alternative Mutationen bei GIST ohne *KIT/PDGFR*A Mutation - Succinatdehydrogenase-Mangel -



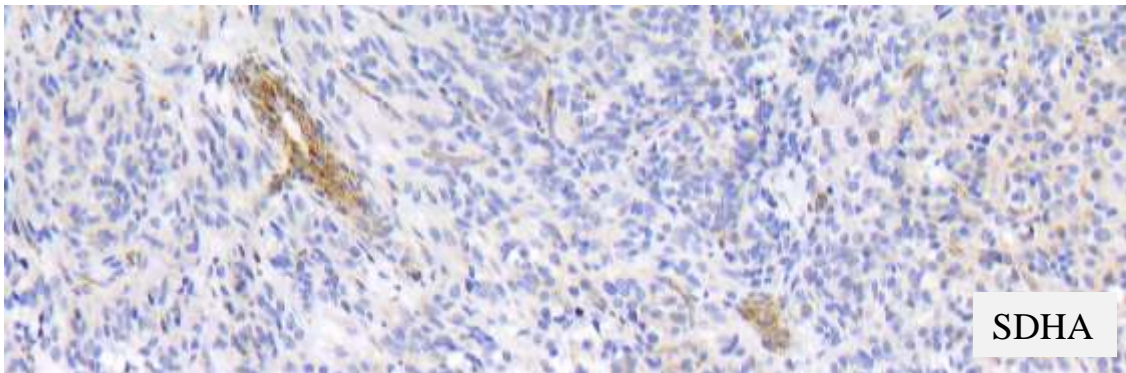
Belinsky et al., *Frontiers in Oncology* 2013

Immunhistochemische SDHB Negativität weist auf einen Defekt im SDH-Komplex hin

auf einen Defekt im SDH-Komplex hin

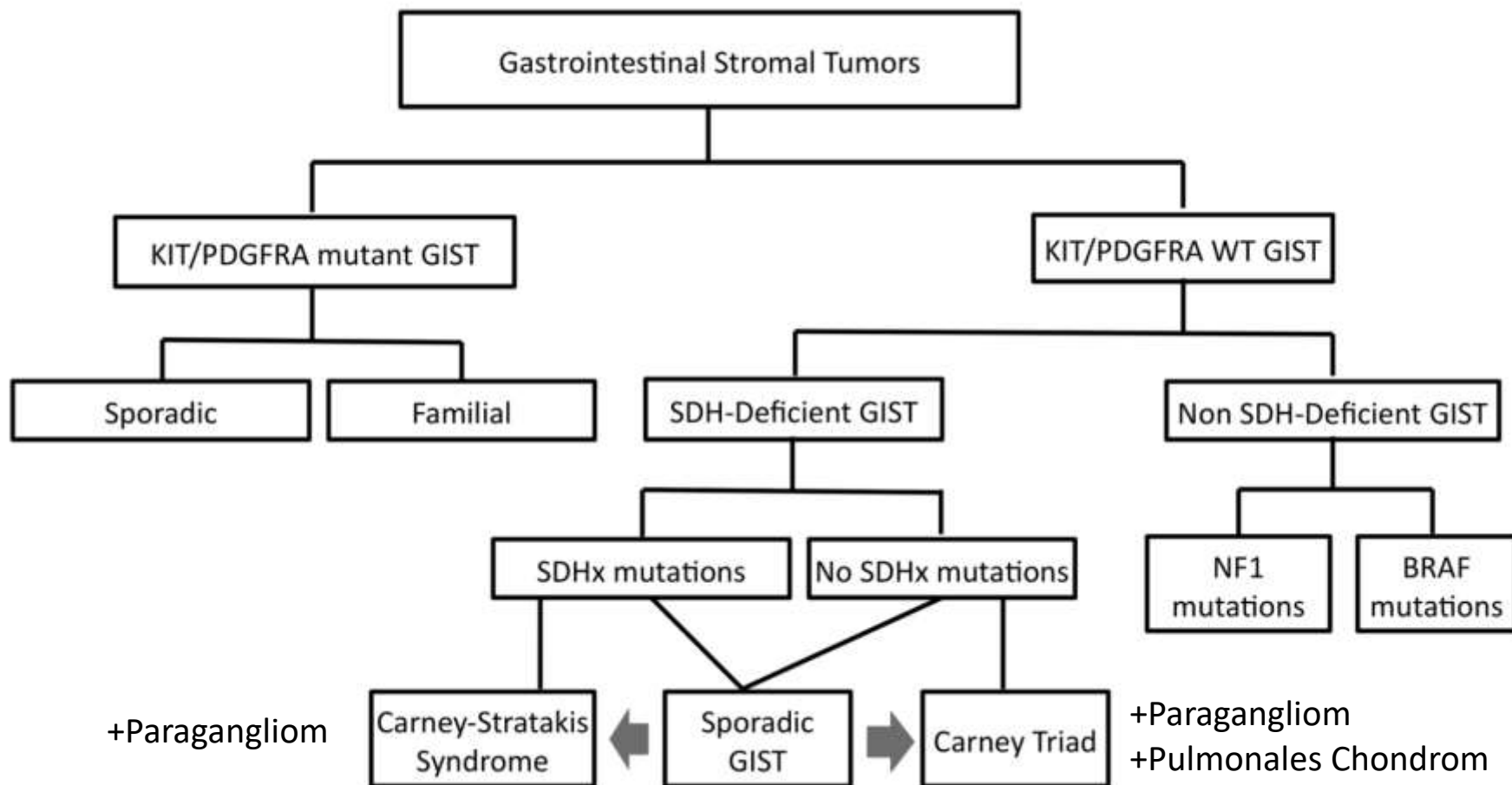


SDHB-Mangel spricht für eine Alteration in egal welchem SDH-Komplexpartner (SDHA/B/C/D)



SDHA-Mangel weist auf eine SDHA Alteration hin

Molekulare Einteilung von GIST



mod. acc. to Boikos and Stratakis, Endocrine 2014

Häufigkeit molekularer Subtypen von GIST

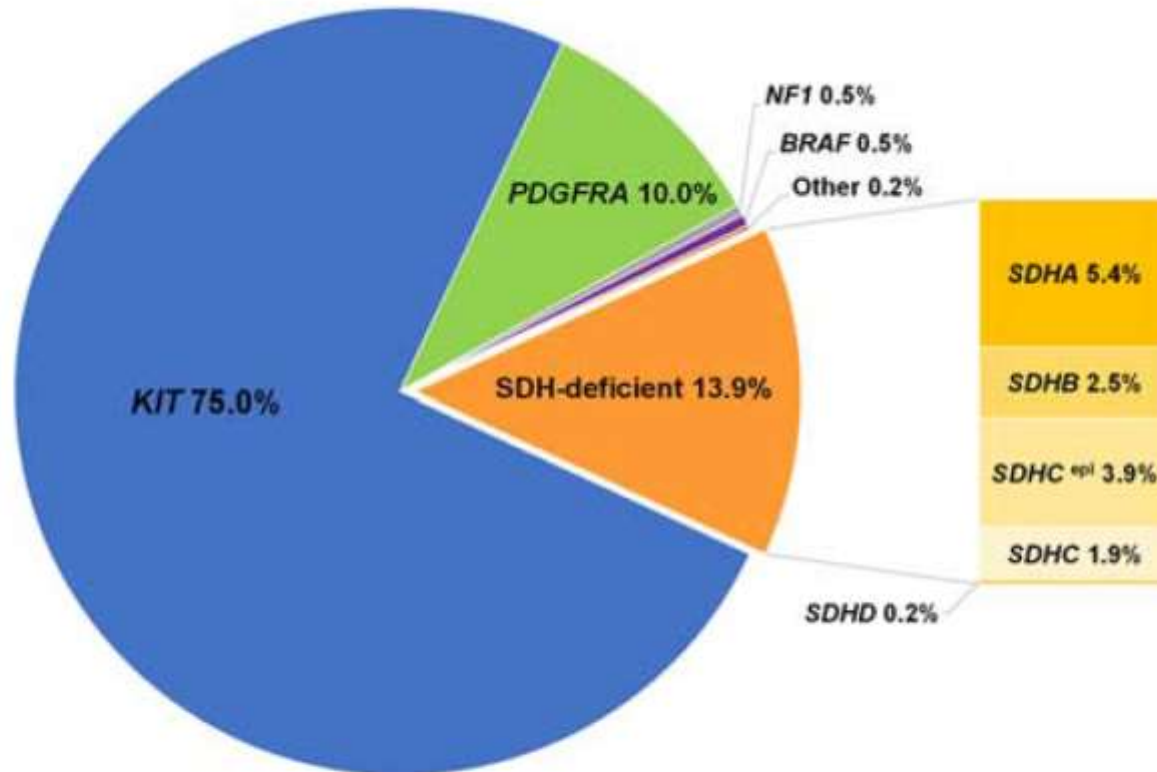


FIGURE 2. Overview of the frequency of the molecular subtypes of gastrointestinal stromal tumor. SDH indicates succinate dehydrogenase.¹⁵ Please see this image in color online.

Schaefer IM et al. Adv Anat Pathol 2017

Schlussfolgerungen

GIST-relevante Gene:

KIT Exons 8, 9, 11, 13, 17

PDGFRA Exons 12, 14, 18

SDHA-D (genetische und epigenetische Alterationen)

KRAS, BRAF

NF1

MAX (Myc associated factor X)

DMD (gene encoding dystrophin)

- andere Gene werden durch die neuen Analyseverfahren mittels NGS folgen



Offene Fragen bei der Mutationsanalyse

- Bei welchen GIST? Abhängig vom Risikoprofil?
- Welche Methode zur Genanalyse ist am besten?
- Zahlt das die Krankenkasse?
- Welche Rolle spielen Untersuchungen am Blut (“liquid biopsy“)?



Das Lebenshaus

Selbsthilfe
GIST

