Molekulare Pathologie in der modernen Diagnostik des Gastrointestinaltraktes

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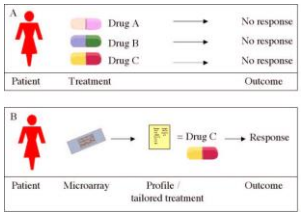
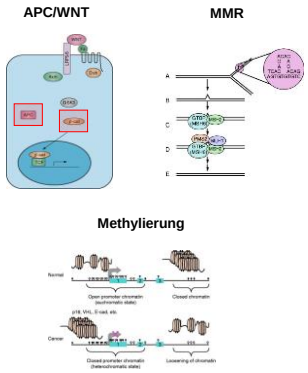
GILAB AG
XXII. Diagnostik Symposium
Schaan, 10.03.2016

- Einleitung
- Technik
- Anwendungen
- NGS
- Allgemeine Schlussfolgerungen

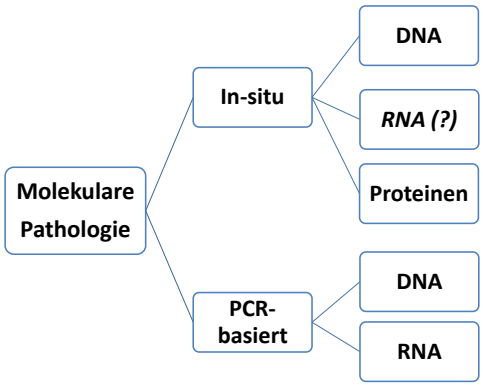


"The right drug, the right dose, for the right patient, at the right time"

Brown et al. 2003



Robbins, 9. Auflage



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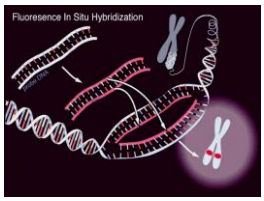

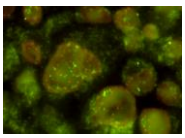

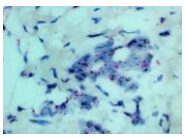

IHC

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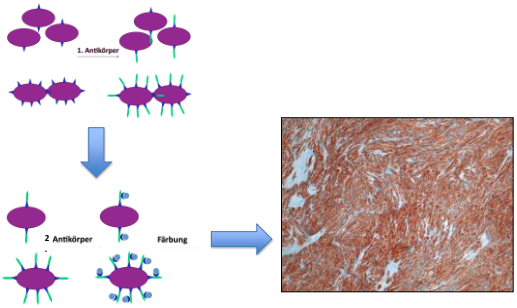

PCR

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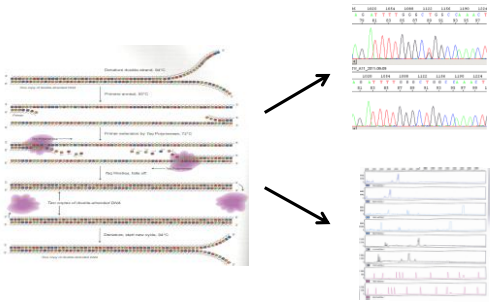

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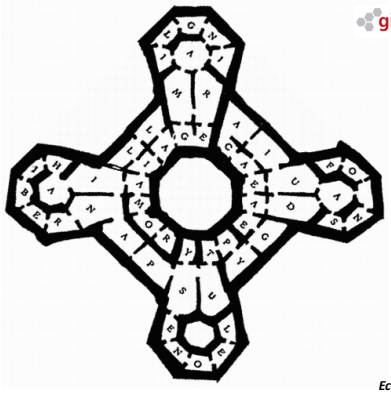

Eco, 1981

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Magenadenokarzinom

WHO 2010

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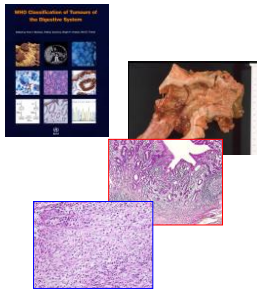
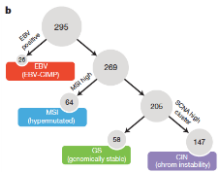
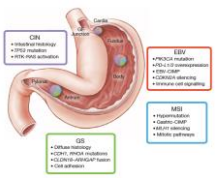
Tab. 2 Klassifikation der Weltgesundheitsorganisation der Karzinome des Magens [13]	
Epitheliale Tumoren	**ICD-O M**
Adenokarzinom	8140/3
1. Papilläres Adenokarzinom	8260/3
2. Tubuläres Adenokarzinom	8211/3
3. Muzinöses Adenokarzinom	8480/3
4. Gering kohäsives Karzinom (einschließlich Siegelringzellkarzinom und anderer Varianten)	8490/3
5. Gemischtes Adenokarzinom	8255/3
Adenosquamöses Karzinom	8560/3
Karzinom mit lymphoidem Stroma (medulläres Karzinom)	8512/3
Hepatoides Adenokarzinom	8576/3
Plattenepithelkarzinom	8070/3
Undifferenziertes Karzinom	8020/3



Magenadenokarzinom: Molekulare Klassifikation





The Cancer Genome Atlas Research Network, Nature, 2014

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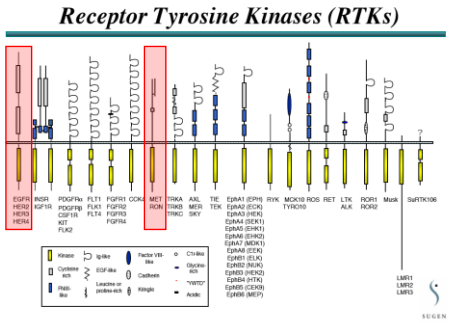
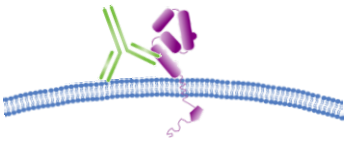
„Protein kinases mediate most of the signal transduction in eukaryotic cells“

Manning et al., 2002

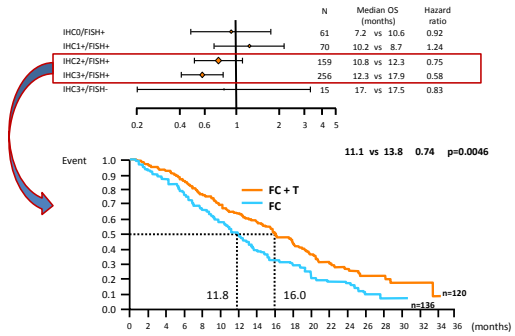


www.kinase.com/kinome

ERBB2 (HER2) und Trastuzumab



- Hemmung HER2-Signalweg in HER2-positive Tumoren
- Hemmung HER2-Activierung durch Blockierung extrazelluläres Domän
- Aktivierung ADCC



Bang YJ et al. Lancet 376: 687-97, 2010

CERBB2 (HER2)

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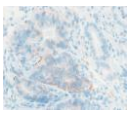
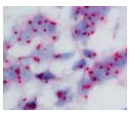
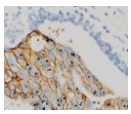
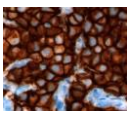
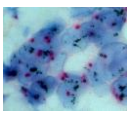
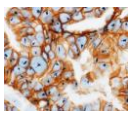
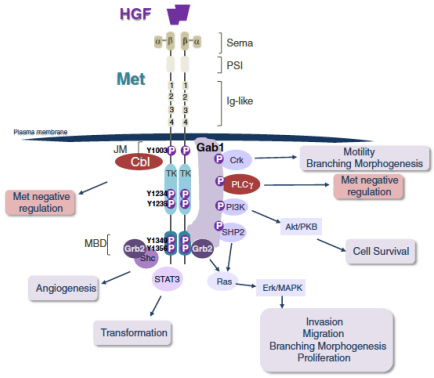
IHC	SISH	GP
		
		

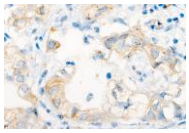
„Vor dem Einsatz einer palliativen medikamentösen Tumortherapie sollte der HER-2-Status...bestimmt werden. Die histopathologische Bestimmung...soll qualitätsgesichert durchgeführt werden“

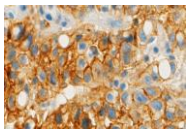
S-3 Leitlinien zum Magenkarzinom, 2013



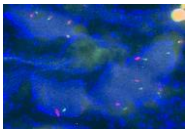
CMET

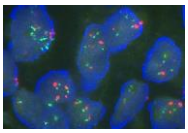
IHC





FISH





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„Eine (neo)adjuvante Therapie mit zielgerichteten Substanzen alleine oder in Kombination mit Chemotherapie soll außerhalb von Studien nicht durchgeführt werden“

S-3 Leitlinien zum Magenkarzinom, 2013

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Schlussfolgerungen I

- **ERBB2 (HER2) wichtig für die Progression vom metastat. MK und mit Antwort zur gezielten Therapie assoziiert**
- **Bestimmung des HER2-Status wichtig im metastatischen Magenkarzinom**
- **Andere RTK in Rahmen von Studien**
- **Molekulare Klassifikation (?)**

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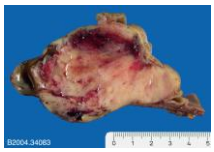
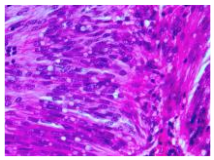
GIST

2001

„Primäre spindelzellige epitheloidzellige, gemischte oder pleomorphe mesenchymale Neoplasien des GI-Trakts“





Agaimy & Schneider-Stock, Pathologe 2010

GIST: Pathogenese

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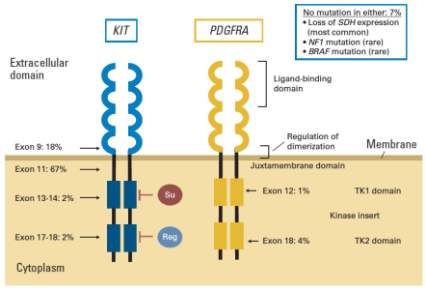

Cioffi & Maki, JCO 2015

GIST: Prognose

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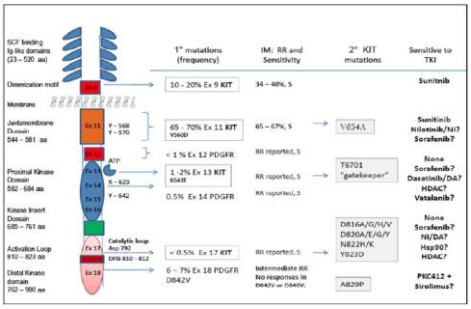
Tumor Parameters		Risk of Progressive Disease[a] (%)			
Mitotic Index (per 50 HPF)	Size (cm)	Gastric	Duodenum	Jejunum/Ileum	Rectum
≤5	≤2	None (0%)	None (0%)	None (0%)	None (0%)
	>2, ≤5	Very low (1.9%)	Low (4.3%)	Low (8.3%)	Low (8.5%)
	>5, ≤10	Low (3.6%)	Moderate (24%)	(Insuff. Data)	(Insuff. Data)
	>10	Moderate (10%)	High (52%)	High (34%)	High (57%)
>5	≤2	None[b]	High[b]	(Insuff. Data)	High (54%)
	>2, ≤5	Moderate (16%)	High (73%)	High (50%)	High (52%)
	>5, ≤10	High (55%)	High (85%)	(Insuff. Data)	(Insuff. Data)
	>10	High (86%)	High (90%)	High (86%)	High (71%)

AFIP, JNCNN, 2007 (sog. “Miettinen’s criteria”)

GIST: RTK-Mutationen

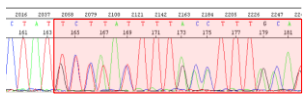
- **Lokalisation**
  - ***KIT* Exon 9 Dünndarm, *PDGFRA* Magen**
- **Histologie**
  - ***PDGFRA* epithelioid, WT epithelioid**
- **Prognose**
  - ***KIT* exon 9 schlecht (?)**
  - ***KIT* exon 11 Deletion schlecht, *PDGFRA* oft indolent**
- **Therapie - Resistenz**
- **Primäre Resistenz (10-20%)**
  - **Ex 9, Ex 13-17,“Wild-Typ“**
- **Sekundäre Resistenz (24 Mo)**
  - **Zweite Mutation (Ex 13-17)**
  - **KRAS?, BRAF?**

GIST: RTK-Mutationen

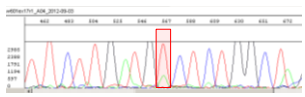


Gounder, Cancer Chemoter Pharmacol 2011

GIST: RTK-Mutationen



p.Ala502-Tyr503dup



p.Asp816Val

Schlussfolgerungen II

- „Gold-standard“ in GIST ist Chirurgie
- RTKI für adjuvante Therapie

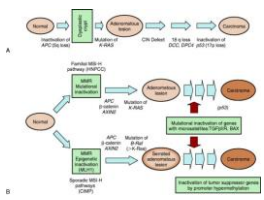
⇩

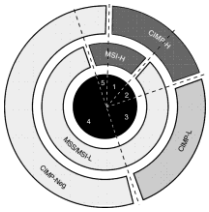
- **Mutationsanalyse immer, wenn adjuvante Therapie angezeigt**

- Die Zukunft: neoadjuvante Therapie ?

Kolonkarzinom

KRK





Robbins, 9. Auflage

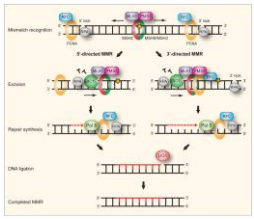
Jass, Histopathology, 2006

KRK: Molekulare Einteilung

- 3 „grobe“ molekulare „pathways“
  - APC-CIN (Adenom-Karzinom Sequenz, FAP)
  - MSI-CS (Lynch, Epigenetik)
  - CIMP (Epigenetik)
- Prädiktive Faktoren
  - G-proteins, PI3KCA

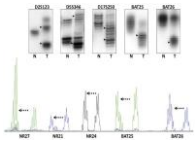
MMR

- Ungepaarte Nukleotiden
- Insertion-Deletion loops
- Komplexer „Apparat“



MSI

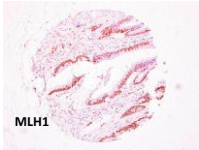
- Kurze Sequenzen ncDNA
  - Mono- , Di-, Trinukleotiden
    - AAAAA..., CACACA..., CAGCAGCAG
- Bethesda Panel
  - 5 Marker
  - ≥2 Instabil

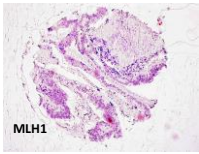


MMR

- IHC
  - MLH1, MSH2, MSH6, PMS2
  - Sensitivität 92.5%
  - Specifität 100%
  - PNW 96.7%
  - PPW 100%

Lindor et al., JCO 2002

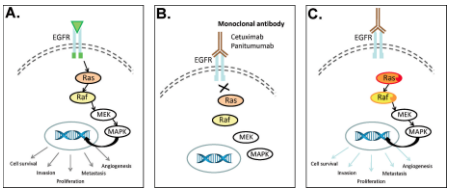




MMR IHC: Bedeutung

MLH1	MSH2	MSH6	PMS2		
+	+	+	+	MSS	=
-	+	+	-	MSI	Sporadisch od. Germline
+	-	-	+	MSI	Germline
+	+	-	+	MSI	Germline
+	+	+	-	MSI	Germline

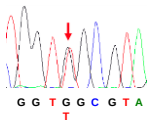
KRK: gezielte Therapie



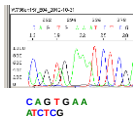
Bedeir, APLM 2013

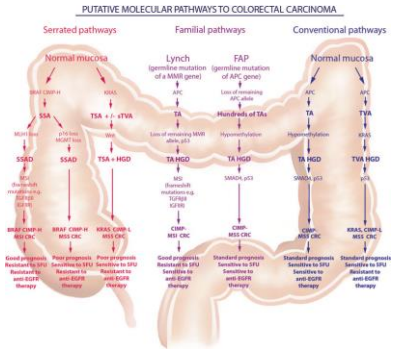
KRK: gezielte Therapie

- RAS
  - 30-40% der Fälle
  - Negativer prediktiver Wert für anti-EGFR
  - Subset prognostischer Wert (G13D) und immunogen

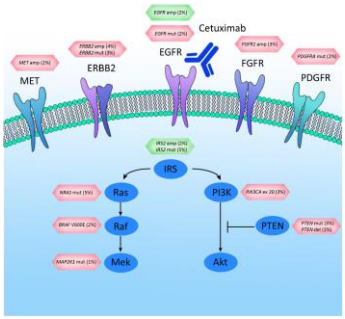


- BRAF
  - 10-15%
  - Negativer predictiver Wert für anti-EGFR
  - Serrated pathway (MSI sporadisch)
  - Schlechtere Prognose





Bettington, Histopathology, 2013

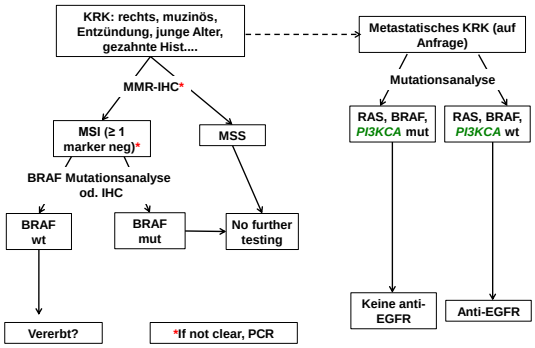


Bertotti et al., Nature 2015

KRK: Molekulare Klassifikation

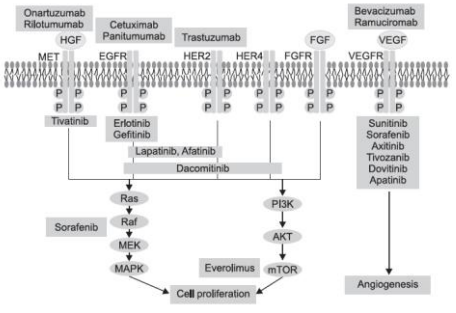
CMS1 MSI immune	CMS2 Canonical	CMS3 Metabolic	CMS4 Mesenchymal
14%	37%	13%	23%
MSI, CIMP high, hypermutation	SCNA high	Mixed MSI status, SCNA low, CIMP low	SCNA high
BRAF mutations		*KRAS* mutations	
Immune infiltration and activation	WNT and MYC activation	Metabolic deregulation	Stromal infiltration, TGF-β activation, angiogenesis
Worse survival after relapse			Worse relapse-free and overall survival

Guinney et al., Nat Med, 2015



Schlussfolgerungen III

- **MSI-H BRAFmut sporadisch**
- **MSI-H Immunogen**
- **RAS Mutationen Exonen 2,3,4 prädiktiv (anti-EGFR)**
- **RAS Mutationen Exone 2,3,4 UND BRAF Mutationen (V600E) prognostisch**
- **PIK3CA (Exon 9 und 20) prädiktiv (Aspirin UND Anti-EGFR)**



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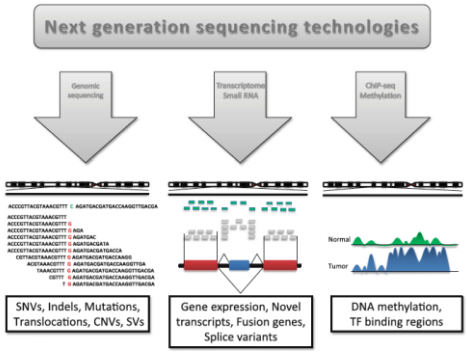
„Normal human genomes are all alike, but every cancer genome is abnormal in its own way.“

Meyerson et al., Nat Rev Gen 2010



Schweiger, Cancer Met Rev 2010

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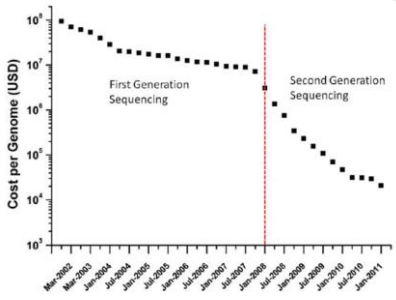

Niedringhaus et al., Anal Chem 2009

Was und Wie Sequenzieren?

- „Actionable“ Mutationen
  - Billig, klein, einfach
- Prognostisch
  - HER2 in Brust
- Prädiktiv
  - KRAS in Kolon
- Panels auch mit Markers in Trials

ABL1	EZH2	JAK3	PTEN
AKT1	FBXW7	IDH2	PTPN11
ALK	FGFR1	KDR	RB1
APC	FGFR2	KIT	RET
ATM	FGFR3	KRAS	SMAD4
BRAF	FLT3	MET	SMARCB1
CDH1	GNA11	MLH1	SMO
CDKN2A	GNAS	MPL	SRC
CSF1R	GNAQ	NOTCH1	STK11
CTNNB1	HNF1A	NPM1	TP53
EGFR	HRAS	NRAS	VHL
ERBB2	IDH1	PDGFRA	
ERBB4	JAK2	PIK3CA	

Beadling, J Mol Diagn, 2013

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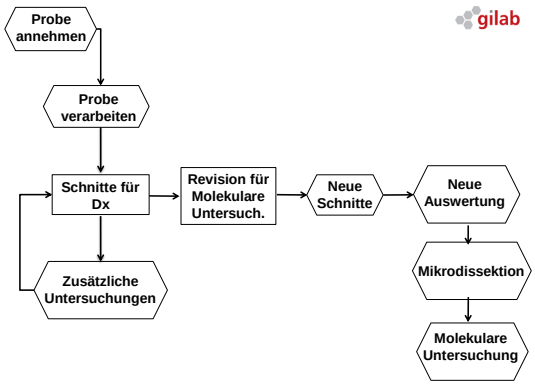

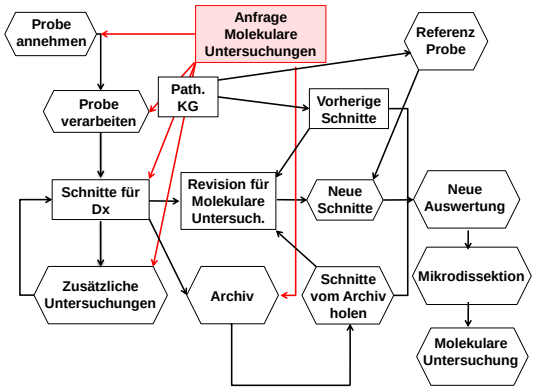

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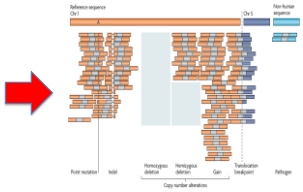

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