all-RAS und BRAF testing beim Colonkarzinom: state oft the art 2015

Wolfgang Hulla
PBI, KFJ-Spital, Wien

RAS Status

- Grundlagen
- Methoden
- QA

…. von 2008 bis 2015

KRAS mutation testing for predicting response to anti-EGFR therapy for colorectal carcinoma: proposal for an European quality assurance program

- J. H. J. M. van Krieken & A. Jung & T. Kirchner & F. Carneiro & R. Seruca & F. T. Bosman & P. Quirke & J. F. Fléjou & T. Plato Hansen & G. de Hertogh & P. Jares & C. Langner & G. Hoefler & M. Ligtenberg & D. Tiniakos & S. Tejpar & G. Bevilacqua & A. Ensari
  - Virchows Arch (2008) 453:417–431

Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials.

- M. J. Sorich, M. D. Wiese, A. Rowland, G. Kichenadasse, R. A. McKinnon & C. S. Karapetis
  - Annals of Oncology 26: 13–21, 2015

2008

- Colorectal carcinoma
- Anti-EGFR therapy
- KRAS mutation testing
- Practice guidelines
- Quality assurance

- KRAS mutation testing
  - Methoden
- Practice guidelines
  - Prä-Analytik
  - Befundung
- Quality assurance
  - Interne Qualitäts-sicherung
  - ESP **Colon External Quality Assessment Scheme**

2013

- Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer.
  - Douillard JY, Oliner KS et al., N Engl J Med. 2013 Sep 12;369(11):1023-34.
- CONCLUSIONS:
  Additional RAS mutations predicted a lack of response in patients who received panitumumab-FOLFOX4. In patients who had metastatic colorectal cancer without RAS mutations, improvements in overall survival were observed with panitumumab-FOLFOX4 therapy.
  - EU LABEL: Vectibix (09/2013) / Erbitux (01/2014) is indicated for the treatment of adult patients with wild-type RAS metastatic colorectal cancer.

2015

- Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials.
  - Systematic review and meta-analysis of nine RCTs comprising a total of 5948 participants
  - “Approximately 20% of KRAS exon 2 wild-type tumors harbored one of the new RAS mutations.”
  - "True" wild-type RAS mutations had significantly superior progression-free survival (PFS) ($P < .001$) and overall survival (OS) ($P = .008$) with use of anti-EGFR monoclonal antibodies (mAbs).
    - Sorich Ann Oncol. 2015 Jan;26(1):13-21.

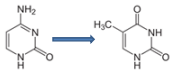
2015

- RAS mutation
- pharmacogenomics
- Cetuximab
- Panitumumab
- predictive biomarker
- meta-analysis

- all-RAS mutation testing
  - Test-Algorithmen
  - Methoden
- Practice guidelines
  - Prä-Analytik
  - Befundung
- Quality assurance
  - Interne Qualitäts-sicherung
  - ESP
    Colon External Quality Assessment Scheme

Prä-Analytik

- Primärmaterial
  Problem: Op nach neo-adjuvanter Therapie
- Fixierung
  CAVE: Cytosindesaminierung oder FREITAG!!!

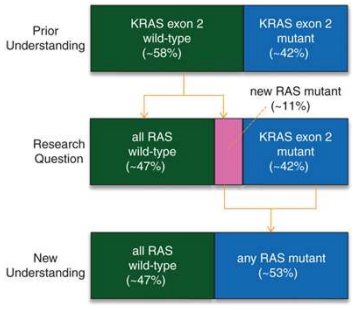


- Dokumentierte Gewebeselektion
- DNA-Präparation
  Quantifizierung
  Qualitätsbestimmung

RAS-testing / PBI-KFJ

1. cobas® KRAS Mutation Test
   – Identifiziert Mutationen in den codons 12, 13 und 61 des KRAS Gens
   – cobas® 480 System - CE-IVD Zertifizierung
2. LightMix® Kit BRAF V600
3. KRAS+ und NRAS Mutations-Analyse
   – PCR und Dideoxy-Sequenz-Analyse
   – RAS LightMix® Kit TIBMOLBIOL
   – KRAS and NRAS Mutation Detection Kits AmoyDX

Grouping of tumors by KRAS exon 2 mutations and extended RAS mutations.



Sorich M J et al. Ann Oncol 2015;26:13-21

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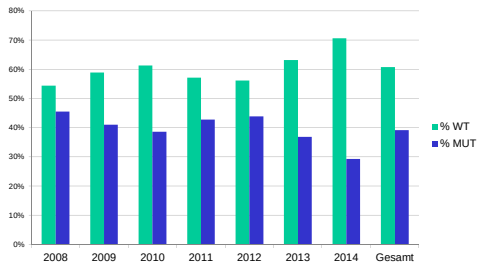
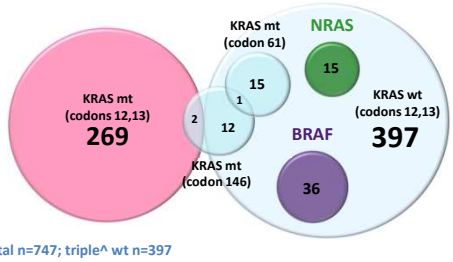
KRAS Mutation / PBI-KFJ



What we know about mutations in the EGFR pathway



Total n=747; triple^ wt n=397

Adapted from De Roock W, et al. Lancet Oncol 2010;11:753–762

aktivierende BRAF-Mutation

- T1799A mutation (V600E)
- seit 2009
- cobas® 4800 BRAF V600 Mutation Test
- Validierung durch nationale Ringversuche
- secondary method needed
  clamped-PCR und Schmelzkurven-Analyse
  (LightMix® Kit BRAF V600 TIBMOLBIOL)

PBI / KFJ

KRAS WT - BRAF exon2 Mutation (ab 2013)

Jahr	Anzahl der Bestimmungen	WT	MUT		
2013	120	105	15	12,5%	7,9%
2014	148	133	15	10,5%	7,1%

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Methoden

- Sanger Sequenzierung
- Allel-spezifische realtime-PCR
- Pyro-Sequenzierung
- Reverse Hybridisierung
- NGS
- Liquid biopsy
- Gold-Standard ev. LNA/PNA
- RAS LightMix® Kit (LNA) ARMS
- PyroMark System
- StripAssay, LCD-Array
- BEAMing Digital PCR

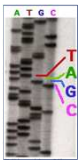
ÖGP 2014 PBI, KFJ-Spital, Wien

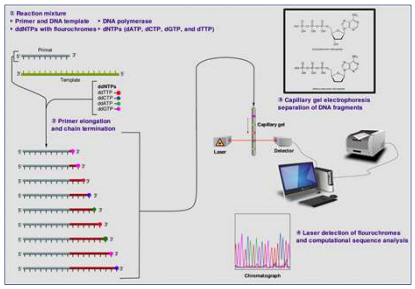
all-RAS Mutationsanalyse

- DNA-Sequenz-Analyse
  + sämtliche Mutationen (neue Mutationen ?)
  - Sensititivität
  - aufwendig
- Allel-spezifische Realtime-PCR
  + hohe Sensitivität und Spezifität
  + einfach
  - neue Mutationen (Validierung ?)
- PCR und reverse Hybridisierung
- NGS (MPS)

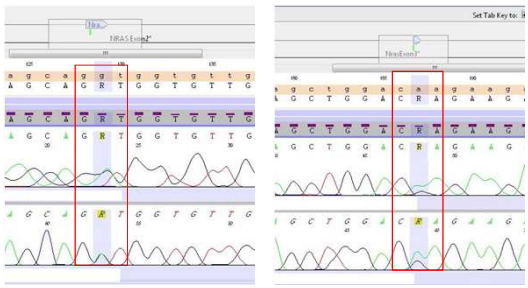
Dideoxy-sequencing

10ng DNA / rxn



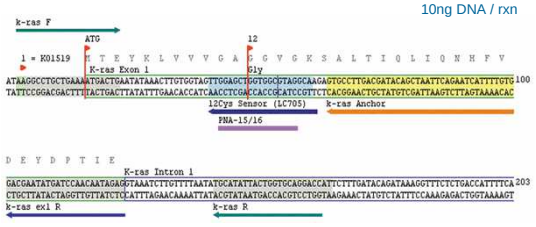


NRAS exon2 & 3 Mutation



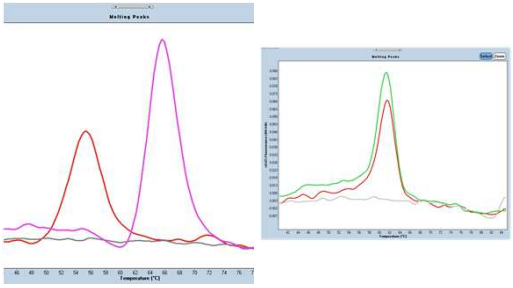
Allele-specific realtime-PCR I

10ng DNA / rxn

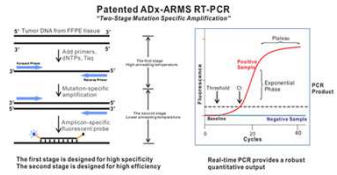


WT-clamped-PCR and hyb-probes

NRAS exon2 & 3 Mutation

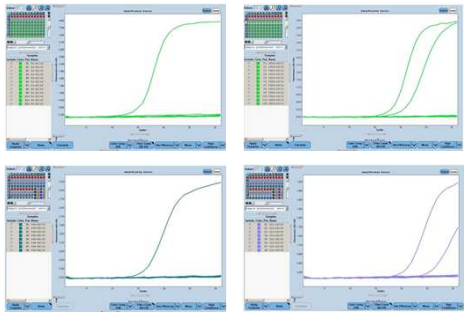


Allele-specific realtime-PCR II



- ARMS
- 10 ng DNA / rxn
- One tube – two temperature profiles
- First round of amplification - high specificity, low efficiency amplification
- Second stage at lower annealig temperature - also highly specific, amplifying only target sequences, but also very efficient. Quantification

KRAS und NRAS Mutationen



Befundung

- Molekularpathologischer Befund MP Beispiel/2015

Im vorliegenden Untersuchungsmaterial eine aktivierende Mutation im Codon 12/13 des KRAS-Gens NACHWEISBAR.

- ad Histologischer Befund Beispiel/2015
- Molekularpathologische Befunde MP KRAS/2015, MP RESTRAS/2015 und MP BRAF/2015:

In Zusammmenschau der molekularpahtologischen Befunde ist eine aktivierende Mutation der KRAS- und NRAS-Gene (Exons 2, 3 und 4) sowie des BRAF-Gens im vorliegenden Untersuchungsmaterial NICHT nachweisbar.

- Anmerkungen

WICHTIG:

Hochgradig eingeschränkte Aussagekraft des negativen KRAS-Mutations-Status aufgrund des äußerst geringen Anteils neoplastischer Zellen (<<10%) im vorliegenden Material. Neueinsendung von bioptischem oder Operations-Material in Anbetracht möglicher therapeutischer Optionen empfohlen.

RAS-testing 2014

RAS-Statistik 2014							
		Anzahl der Bestimmungen	WT		MUT		
	KRAS	211	149	70,6%	62	29,4%	
	BRAF	148	133	89,9%	15	10,1%	
	RAS+	131	115	87,7%	16	12,2%	
	RAS		115	54,5%	88	41,7%	

Prevalence of new RAS mutations across studies.

	New RAS Total[a]	KRAS Exon 3[a]	KRAS Exon 4[a]	NRAS Exon 2[a]	NRAS Exon 3[a]	NRAS Exon 4[a]
OPUS	26.3%	5.9%	9.3%	6.8%	5.1%	0.8%
PICCOLO	9.8%	NR[b]	3.7%[c]	6.3%[d]	NR[b]	NE
20020408	17.6%	4.8%[b]	5.0%	4.2%	3.0%[b]	1.1%
20050181	20.5%	4.6%	7.9%	2.3%	5.8%	0.0%
PRIME	17.4%	3.7%[b]	5.6%	3.4%	4.1%[b]	0.0%
FIRE-3	16.0%	4.3%[b]	4.9%[c]	3.8%	2.0%[b]	0.0%
PEAK	20.1%	4.1%	7.7%	5.4%	5.9%	0.0%
COIN	8.4%	2.1%[b]	NE	0.9%[d]	3.0%[b]	NE
CRYSTAL	14.7%	3.3%	5.6%	3.5%	2.8%	0.9%
SUMMARY[f]	19.9% (16.7%, 23.4%)	4.3% (3.3%, 5.5%)	6.7% (5.7%, 7.9%)	3.8% (3.0%, 4.8%)	4.8% (3.4%, 6.8%)	0.5% (0.2%, 1.2%)

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QS

- Primär Material und Anteil neoplastischer Zellen
  - <<10% : „der negative Befund nicht ausreichend aussagekräftig, wegen therapeutischer Konsequenz Neueinsendung empfohlen“
- DNA-Qualität
- Methoden Auswahl und Evaluierung
- Statistische Untersuchungen
- EQA

Main recommendations for RAS testing

- Network arrangements should be established to ensure rapid and robust tissue pathways from referral centres to testing laboratories.
- Either primary or metastatic CRC tissue can be used for RAS testing.
- Either biopsy or resection specimen tissue can be used for RAS testing
- The minimum neoplastic cell content tested should be at least two times the assay's LOD.
- RAS analysis should include at least KRAS codons 12, 13, 59, 61, 117 and 146 and NRAS codons 12, 13, 59 and 61.
- Turnaround time for RAS testing (of the above panel) should be ≤7 working days from receipt of the specimen in the testing laboratory to issuing of the final report, for >90% of specimens.

J Clin Pathol doi:10.1136/jclinpath-2014-202467

Main recommendations for RAS testing

- Validation (or verification, where more applicable) of RAS testing assays should be performed and recorded prior to implementation in clinical use.
- The minimum controls needed for RAS testing should be mutant, wild-type and non-template controls for each region/amplicon analysed.
- Laboratories should audit their results to ensure that the proportion of mutant cases for each gene and codon are in line with published data. If a significant deviation is seen, the performance of the assay (from specimen reception to reporting of results) should be investigated.
- Laboratories should make every possible effort to reduce failure rates, including reviewing the quantity and quality of DNA obtained from routine specimens.
- Laboratories providing RAS testing of CRC should demonstrate successful participation in a relevant EQA scheme, and be appropriately accredited.

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molpath-Mastermix

- Wolfgang Hulla
- Martina Bergmann
- Sandra Gröller
- Tamara Kalchbrenner
- Harald Kirschner
- Martin Klimpfinger

PBI, KFJ-Spital, Wien