**EGFR and K-RAS mutation analysis**

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Increasing evidence points to the mutational status of EGFR and K-ras genes as effective molecular predictors for prediction of responsiveness and efficacy of EGFR-targeted therapies (moAb or TKIs).

**Mechanisms of Inhibiting the EGFR Axis**

Inhibition Strategies:  
- EGFR-TK inhibitors  
- Anti-EGFR mAb

**Prediction of responsiveness and efficacy of EGFR-targeted therapies (moAb or TKIs)**

-> Increasing evidence points to the mutational status of EGFR and K-ras genes as effective molecular predictors.
As a consequence, mutational screening tests for K-ras and EGFR may provide a direct and valuable guidance for clinicians to make decision on EGFR-targeted therapies.

Current indications (January 2010; may change in time)
1) EGFR x19/x21 -> EGFR-TKI lung cancer
2) K-ras -> contra anti-EGFR moAb colorectal cancer

Mutational analysis
Requirements for use in clinical / diagnostic setting include, amongst others:
- validated assay
- sufficient sensitivity
  limiting factors:
  - paraffin material (cross-linked and/or fragmented DNA)
  - tumor cell % -> enrichment by macro/micro-dissection
  - limiting amount of material (biopsies/cytology)
- high-throughput / non time-consuming
- cost-effective
Variety of assays

In-house assays and commercial assays
- (nested-) PCR following cycle sequencing
- fragment analysis / RFLP
- SNaPshot primer extension assay
- point-EXACCT
- real time PCR
- melting analysis
- PCR followed by strip hybridisation

Some of which will be discussed below.
High Resolution Melting (HRM)

The melting curve of a PCR product depends on o.a.
- GC content
- length
- sequence
- heterozygosity

-> Different genetic sequences melt at slightly different rates

Comparing the melting curve of a specimen to a reference (wild type) allows scanning for any sequence variation

-> Pre-screen assay with possibility to sequence PCR products to confirm genotype

HRM EGFR/K-ras panel

Panel of HRM assays to prescreen for mutations:

- K-ras x1  -> a.o. codon 12/13
- K-ras x2  -> a.o. codon 61
- EGFR x19 -> a.o. deletions
- EGFR x20p -> a.o. ins 770/771
- EGFR x20d -> a.o. T790M
- EGFR x21  -> a.o. L858R

Kramer et al. 2009
Heideman et al. 2009
**Procedure of mutation detection by HRM**

1) run cycling and melting program
2) check for correct amplification curves
3) check for melting curves and melting peaks (amplimer area and probe area, if applicable)
4) evaluate normalized, temperature-shifted difference plot (amplimer)

**Analytical performance:**
High analytical sensitivity of HRM and subsequent sequencing

*example: K-ras exon 1 (G12C) 2.5-5% mutant DNA in a background of wt can be well discriminated from background/noise signal*

*Kramer et al. Cell Oncol 2009*

**Performance of HRM on DNA isolated from FFPE-tissue in comparison to conventional nested-PCR/sequencing**

1. High genotype agreement of HRM following sequencing with conventional nested-PCR following cycle-sequencing (kappa > 0.95)
2. Less-test failures
3. More mutations detected by HRM (likely related to the higher sensitivity as compared to conventional assay)

*Kramer et al. 2009
Heideman et al. 2009*

**K-ras: high genotyping agreement of HRM with conventional cycle sequencing assay**

*Overall agreement in genotyping: kappa values of 99.3%*

*Kramer et al. 2009*
Conclusion

Increased interest in mutation analysis in MD in PA

Current indications (January 2010; may change in time)
1) EGFR x19/x21 -> EGFR-TKI lung cancer
2) K-ras -> contra anti-EGFR moAb colorectal cancer

Novel indications likely (b-raf, PIK3CA, ....)

Variety of assays available

Molecular assay useful in clinical/ diagnostic setting
- applicable to routine material (FFPE / cytology)
- sensitive: low-abundant mutants detectable
- fast and accurate
- prescreen technique -> high throughput
- allows for genotyping

EGFR: high genotyping agreement of HRM with conventional cycle sequencing assay

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Heideman et al. 2009

Acknowledgements

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