Mutatie analyse m.b.v. Taqman techniek

Judith Bovée & Tom van Wezel
Leiden University Medical Center
The Netherlands

Molecular Tumor Diagnostics

- Mutation
- Methylation
- Amplification
- Translocation

- Faster
- Increased sensitivity
- Minimal amounts of tissue
- Preoperative
EGFR mutation analysis in NSCLC

**EGFR mutation positive**
- anti EGFR therapy
- Median PFS: 9.5 vs 6.3 mon
- Chemo therapy

**EGFR mutation negative**
- anti EGFR therapy
- Median PFS: 1.5 vs 5.5
- Chemo therapy

Accurate and rapid molecular diagnosis is pivotal!


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Preoperative Staging: Cytology

- Current lung cancer staging guidelines acknowledge:
  - Endosonography with fine needle aspiration
    - Mediastinal lymph nodes
  - Minimally invasive alternative to surgical staging to detect nodal disease.
  - Minimal material

Pathological diagnosis: On Cytology from lymph nodes
Molecular Pathology on the same material: Option for Personalized medicine?


Mediastinoscopy vs Endosonography for Mediastinal Nodal Staging of Lung Cancer
A Randomized Trial
Samples

Mediastinal lymph node

Metastasis, resection

Tissue preparation
Sanger sequencing

• Although gold standard: Disadvantages
  - Turnover time
  - WGA may be needed
  - HR-MCA is not applied
  - Sensitivity +/- 10% mutant allele

• Many mutations are “hotspot”
• Why sequence >100 bases if 4 is enough... or 1...

Hydrolysis Probes Assays

• Taq Polymerase + Paqman= Taqman

TaqMan

From Wikipedia, the free encyclopedia

**TaqMan** probes are hydrolysis probes that are designed to increase the specificity of real-time PCR assays. The method was first reported in 1991 by researchers at Cetus Corporation and the technology was subsequently developed by Roche Molecular Diagnostics for diagnostic assays and by Applied Biosystems for research applications.

The TaqMan probe principle relies on the 5’→3’ exonuclease activity of Taq polymerase to cleave a dual-labeled probe during hybridization to the complementary target sequence and fluorophore-based detection. As in other real-time PCR methods, the resulting fluorescence signal permits quantitative measurements of the accumulation of the product during the exponential stages of the PCR; however, the TaqMan probe significantly increases the specificity of the detection. TaqMan probes were named after the videogame *PacMan* (Taq Polymerase + Paqman = TaqMan) as its mechanism is based on the *PacMan* principle.
Allele specific taqman probes

Hydrolysis Probes Assays

As long as the fluorophore and the quencher are in proximity, quenching inhibits any fluorescence signals. 5’ to 3’ exonuclease activity of the polymerase degrades the probe that has annealed to the template.
General approach

• Standard hydrolysis probes assay’s

– EGFR del19... different approach

Rapid Mutation Analysis of Fine Needle Aspirates using allele-specific qPCR

• EGFR: p.L858R*, exon 19 deletions*
• BRAF: p.V600E, (p.V600K)
• NRAS, HRAS, KIT, IDH1/2, ....

R. van Eijk et al PlosONE 2011: 6 (3) e17791
Implementation in Molecular Diagnostics

- **BRAF** p.V600E
- **BRAF** p.V600K
- **EGFR** p.L858R
- **EGFR** exon 19 deletions
- **KRAS**
  - Codon 12, 13 → 9 assays
- **PIK3CA** p.E542K
- **PIK3CA** p.E545K
- **PIK3CA** p.H1047R
- **NRAS**
  - Codon 12, 61 → 5 assays
- **GNAQ** p.Q209L
- **GNAQ** p.Q209P
- **IDH1** p.R132H
- **IDH1** p.R132C

21 Patient samples
3 controls
10 µL qPCR

* Yung et al, Clin Can Res 15/2016

Analysis through Excel spreadsheet

(FAM(Mut)-(y * minimal of 3 water)) / (VIC(WT)-(y * minimal of 3 water))

(Log (FAM(Mut)control / VIC(WT)control)) / (Log (FAM(Mut)Test/VIC(WT)Test))
Example: EGFR L858R

And it works on minimal input DNA

Effect of the DNA concentration on the c.34G>T KRAS assay.

R. van Eijk et al PlosONE 2011: 6 (3) e17791
EGFR deletion exon 19

- 'Classical detection'

```
80  80  100  110
```

Taqman assay for exon 19 deletions

Wild type signal is lost if a deletion is present
• 43 NSCLC cases: cytology and histology
• discordance rate only 0.20% (1 KRAS mutation found in metastasis (Cyto) was absent in a primary tumor)
• Microdissection is essential to obtain high tumor cell percentages (>50%) to detect EGFR exon 19 deletions

Potential pitfalls: restricted to selected hotspots

• V600E Specific Taqman assay
  – V600E + or -

• COBAS
  – V600+ or V600-

V600E 75-90%, V600K < 20%
Taqman (BRAF V600K)

Whole slide; 5-10% tumor

Potential pitfalls: heterogeneity

In spite of the high tumor % mutant allele is low!

Heterogeneity within metastasis
Genomic imbalance?

Microdissection; 80-90%
Intra- and Inter-Tumor Heterogeneity of BRAF<sup>V600E</sup> Mutations in Primary and Metastatic Melanoma

Molly Yamamoto<sup>1</sup>, Adam Litteken<sup>1</sup>, Joanne Yoann<sup>1</sup>, Elize Ng<sup>1</sup>, Richard L. Shapiro<sup>1</sup>, Russell S. Bernard<sup>1</sup>, Anne C. Pavlick<sup>1</sup>, Farhad Darvishian<sup>2</sup>, Paul Christos<sup>3</sup>, Matha Mazzoulet<sup>4</sup>, Iman Osman<sup>1</sup>, David Pethel<sup>1</sup>.

Table A: BRAF mutation concordance between primary and metastatic specimens using MS-FASE.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Primary Tumor</th>
<th>Metastatic Tumor</th>
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<tbody>
<tr>
<td>1</td>
<td>Wild Type</td>
<td>Mutant</td>
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</tr>
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<tr>
<td>9</td>
<td>Wild Type</td>
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</tbody>
</table>

IDH: sensitivity

Pansuriya et al Nat Genet 2011
Concluding remarks

• Tumor percentage
  – must be high, microdissection!
• Tumor heterogeneity:
  – Multiple samples where possible
• Input
  – Possible on very low number of tumor cells
  – BUT: preferred on 1000s of tumor cells
• High volume, short turnaround

www.scivee.tv/node/39348

Department of Pathology
- Tom van Wezel
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- Rentsia van Schaik-Ouwersloot
- Sietse van Tol-Rensen
- Soheila Fallahi
- Sandra Uijee
- Karin Kleiverda
- Patrick Lechevall
- Brandt Meylis

T.van_Wezel@lumc.nl