Longcarcinoom nieuwe wegen nieuwe kwaliteitsaspecten

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LUNG CANCER Diagnostic, Predictive flow

Cancer in the lung: primary vs metastases

Primary lung cancer: NSCLC – SCLC
Staging M+

NSCLC:
adenocarcinoma vs squamous cell carcinoma

Adenocarcinoma
KRAS / EGFR mutation
KRAS and EGFR negative:
ALK
BRAF?
PIC3CA?

Squamous cell carcinoma
FGFR1?
DDR2?
PIC3CA?

Erik Thunnissen WCLC2011
Cancer in the lung: primary vs metastases

• Clinical information essential:
• PRIMARY LUNG TUMOR: Surfactant prot A, Napsin A, TTF1
• Saving of material not to do additional stains for metastases:
• Colorectal : CK7, CK20, CDX2,
• Prostate: PSA, PAP,
• Breast: ER, PR, GCDFP15, GATA3
• Germ cell: PLAP, AFPHcG, CD30, OCT3/4, Sox2, Sox17
• Melanocyte: Melan A, HMB45, Sox 10, MITF
• Mesothelium: Calretinin, CK5/6, D2-40, WT-1
• Kidney: RCC, CD10, Pax2, Pax8
• Ovary: CA125, Pax5, Pax 8,
Primary lung cancer: NSCLC – SCLC

- SCLC: CD56, CHROMOGGRANIN, SYNAPTOPHYSIN
- DD SCLC: CD45, KI67

- IN 5% CASES NO DISTINCTION POSSIBLE: BIOLOGY IS NOT BLACK AND WHITE
NSCLC: adenocarcinoma vs squamous cell carcinoma
Squamous cell carcinoma

Study: 20% of squamous cell carcinomas were squamoid: IHC TTF1 or mucin +
Biopsy lung tumor
Biopsy lung tumor

Favour squamous cell carcinoma
Favour adenocarcinoma
NSCLC: adenocarcinoma vs squamous cell carcinoma

<table>
<thead>
<tr>
<th></th>
<th>p63</th>
<th>TTF1</th>
<th>mucin</th>
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<tbody>
<tr>
<td>Squamous cell carcinoma</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>-/+</td>
<td>+</td>
<td>-</td>
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<td>NOS</td>
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<tr>
<td></td>
<td>+++</td>
<td>+</td>
<td>+</td>
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</tbody>
</table>

- 85-90% favour adenocarcinoma or squamous cell carcinoma,
- remaining Bx NOS; Rx Large cell / Adenosquamous carcinoma
LUNG CANCER Diagnostic flow

Cancer in the lung: primary vs metastases

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Staging M+

NSCLC: adenocarcinoma vs squamous cell carcinoma

Adenocarcinoma
KRAS / EGFR mutation
KRAS and EGFR negative: ALK

Squamous cell carcinoma FGFR1?
LUNG CANCER Diagnosis

Cancer in the lung: primary vs metastases

Primary lung cancer: NSCLC – SCLC
Staging M+

NSCLC: adenocarcinoma vs squamous cell carcinoma

prediction

Adenocarcinoma

KRAS / EGFR mutation
KRAS and EGFR negative:
ALK

Squamous cell carcinoma

FGFR1?
“Lazarus Response” to gefitinib: Chemoresistant EGFR mutant adenocarcinoma

January 2002

October 2004

Johnson 2004
EGFR is deregulated in most solid tumors

- Adapated from Rowinsky 2004
EGFR Belongs to the ErbB Family of Cell Surface Receptors

Extracellular Domain

Transmembrane Domain

Intracellular TK Domain

ErbB1 (EGFR)
ErbB2 (HER2/neu)
ErbB3 (HER3)
ErbB4 (HER4)

Amgen slide
Ligand Binding and Dimerization Result in TK Activation

Ligand Binding
- EGF
- TGF\(\alpha\)

High Affinity Binding

Dimerization
- Homodimer
- Heterodimer

ATP

Phosphorylation and Activation
EGFR/HER Family of Surface Tyrosine Kinases

Homodimers
- HER1-HER1
- HER2-HER2
- HER3-HER3
- HER4-HER4

Weak signaling
No signaling

Heterodimers
- HER1-HER2
- HER1-HER3
- HER1-HER4
- HER2-HER3
- HER2-HER4
- HER3-HER4

Strong signaling

EGFR Activation Enhances Pathways Important for Tumor Cell Growth
Mutations in the TK domain of EGFR:

Meta analysis of 5 studies (n=1256)

- Extracellular domain
- Transmembrane region
- ATP binding cleft
- Regulatory domain
- N-lobe
- C-lobe
- TK domain
- P-loop
- αC-helix
- A-loop

Deletions 46%
Duplications/Insertions (9%)
L858R (39%)

Gazdar 2006
Not all EGFR mutations are created equal

“Activating mutations”

“Resistant mutations”

“Indeterminate mutations”

complex mutations = combination of >1 mutation

www.sm-egfr-db

Modified after Riely et al 2006
EGFR mutation

Absent

Present

Mok NEJM 2009, Mitsudomi
Conclusion:
Since in patients without EGFR mutations more harm is done with EGFR-TKI than with chemo-x, EGFR-TKI treatment only for patients with EGFR mutations: selection required.

Mok NEJM 2009, Mitsudomi
### Flow chart NSCLC

<table>
<thead>
<tr>
<th>HE</th>
<th>Squamous cell carcinoma</th>
<th>NOS</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Additional stain</td>
<td>P63 + TTF1 - Mucin -</td>
<td>P63 - TTF1 - Mucin -</td>
<td>P63 - TTF1 + and/or Mucin +</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Squamous cell carcinoma</td>
<td>NOS/ AdSq</td>
<td>Adenocarcinoma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mutation analysis</td>
<td>EGFR/KRAS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EGFR/KRAS</td>
<td></td>
</tr>
</tbody>
</table>
EGFR protein is often over-expressed in NSCLC

- 62% NSCLC
  - 82% squamous cell carcinoma
  - 44% adenocarcinoma
  - 80% adenocarcinoma with BAC features (peripheral adenocarcinomas)

- 0% SCLC

Hirsch et al 2003

Squamous cell carcinoma
**EGFR FISH: Colorado Score System**

**Low copy number**
- EGFR negative

**High copy number**
- EGFR positive

≥ 40% cells with ≥ 4 EGFR signals

Marileila Garcia
**EGFR FISH: Colorado Score System**

- High copy number
- EGFR positive

**Mutant allele specific Amplification**
**Late event**

Marileila Garcia
EGFR mutation analysis: WHO?

Is selection based on clinical grounds sufficient?

- **Non-smokers, Women, Asian**
- Rosell NEJM 2009, 2105 cases 350 mutations
- 68% EGFR mutations in non-smokers; 6% current smokers, 26% ex-smokers
- 73% women, 27% men
- 98% Kaukasian
EGFR mutation analysis: WHO?

- Is selection based on clinical grounds sufficient?
- Non-smokers, Women, Race
- Rosell NEJM 2009, 2105 cases 350 mutations
- 68% EGFR mutations in non-smokers; 6% current smokers, 26% ex-smokers
- 73% women, 27% men
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- **Clinical parameters are insufficient to select patients for EGFR mutation analysis**
WHO?
Histology as triage for EGFR mutation detection?

- Most frequent ADENOCARCINOMAS ~30-10%
- LARGE CELL CARCINOMAS >2%
- Squamous cell carcinomas 1-2%
- Rare
- Small cell carcinoma Rare (combined SCLC-adenocarcinoma)
- Pulmonary salivary gland tumors
WHO?
Histology as triage for EGFR mutation detection?

• Most frequent ADENOCARCINOMAS ~30-10%
• LARGE CELL CARCINOMAS >2%
• Squamous cell carcinomas 1-2%

RARE
• Small cell carcinoma Rare (combined SCLC)
• Pulmonary salivary gland type tumors

• DUTCH guidelines:
  Non-squamous NSCLC
  Not in mucinous AC, LCNEC, carcinoids
Material is limited

• Direct question of pulmonologist/oncologist:
• In case of malignancy EGFR mutation AND EGFR expression?
Molecular pathology:
balance between pulmonology, pathology and molecular biology and oncology

Pulmonologist: clinical information
questions: diagnosis, “EGFR” if malignant

Pathology “EGFR” code: specific handling in contrast to regular

Erik Thunnissen WCLC2011
Pleuravocht
EBUS lymfklier station 7
2010

• Another major breakthrough
### ALK fusion in NSCLC

<table>
<thead>
<tr>
<th>EML4-ALK</th>
<th>TFG-ALK</th>
<th>KIF5B-ALK</th>
</tr>
</thead>
<tbody>
<tr>
<td>E13;A20</td>
<td>E14;A20</td>
<td>E2;A20</td>
</tr>
<tr>
<td>E6;A20</td>
<td>E18;A20</td>
<td>E17;A20</td>
</tr>
<tr>
<td>E20;A20</td>
<td>E15;A20</td>
<td></td>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

**Legend:**
- **Coiled-coil domain**
- **Tyrosine kinase domain**
Tumor Responses to crizotinib, NSCLC with ALK Fusion

\[\% \text{ of best change from baseline}\]

Camidge, DR, et al AACR/IASLC, 2010; Kwak NEJM 2010
Typing – importance treatment consequences

Adenocarcinoma

– Pemetrexed + cisplatin survival benefit over GC
– EGFR - erlotinib/gefinitib
– KRAS, B-Raf
– Alk – crizotinib
– cMET

• Squamous cell carcinoma
  – Gemcitabine + cisplatin (GC)
  – Contra Bevacizumab toxicity
Pathologist

Paraffin block

- vital tumor cells
- necrosis
- stroma
- inflammatory cells

Estimation
% vital tumor cells

➤ 2x Threshold mutation technique
## EGFR Mutation analysis

### Which technique?

<table>
<thead>
<tr>
<th>DETECTION OF</th>
<th>ANALYTICAL SENSITIVITY</th>
<th>SAMPLE TRANSFER[(\times)]</th>
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<tbody>
<tr>
<td><strong>All mutations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR sequencing</td>
<td>20-30%</td>
<td>5</td>
</tr>
<tr>
<td>PCR-HRM/ sequencing</td>
<td>2-5%</td>
<td>2/5</td>
</tr>
<tr>
<td>WAVE Surveyor</td>
<td>(2-?)5%</td>
<td>5</td>
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<tr>
<td>Pyrosequencing</td>
<td>1%</td>
<td></td>
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<tr>
<td>Massive parallel seq.</td>
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<tr>
<td><strong>Only known mutations</strong></td>
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<tr>
<td>SARMS *</td>
<td>0.5-1%</td>
<td>1</td>
</tr>
<tr>
<td>PNA/LNA Clamp</td>
<td>1%</td>
<td>1</td>
</tr>
<tr>
<td>SNAPSHOT (primer extension)</td>
<td>1-5%</td>
<td>5</td>
</tr>
<tr>
<td>PCR Fluorescent RFLP</td>
<td>5%</td>
<td>7</td>
</tr>
<tr>
<td>ME PCR sequencing</td>
<td>0.1%</td>
<td>7</td>
</tr>
<tr>
<td>PCR Invader</td>
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<td>3</td>
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</table>
# EGFR Mutation analysis: Which technique?

**DETECTION OF** | **ANALYTICAL SENSITIVITY** | **SAMPLE TRANSFER**
--- | --- | ---

**All mutations**

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<tr>
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<th>Sensitivity</th>
<th>Transfer</th>
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**Only known mutations**

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</table>

Analytical sensitivity relates to required fraction of tumor cells in sample.

Any of the sensitive methods will do, as long a EQA performance is OK.
ORGANISATIONS guidelines
External Quality Assurance (EQA)

• USA       CAP-AMP-IASLC
• Europe     ESP, ESMO, ETOP, UKNEQAS

• www.EMQN.org
  – Material validated
  – Pilot study (requirement in certified organisation)
  – World wide open 3rd quarter 2011
EQA ring study = proficiency testing

Samples construction ← Central body (certified)
Sample validation ← External laboratory
Participants testing
Report concept
Report final

Thunnissen, 2011 JCP
## Dutch EQA

<table>
<thead>
<tr>
<th>Year</th>
<th>IHC</th>
<th>ISH</th>
<th>Mutation analysis</th>
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<tr>
<td>2008</td>
<td>TMA sections n=17</td>
<td>TMA sections n=17</td>
<td>isolated DNA n=3 from cell lines paraffin sections n=2</td>
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<td></td>
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<tr>
<td>2009</td>
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<td>TMA sections n=13</td>
<td>isolated DNA n=4 from cell lines TMA n=13</td>
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</table>
## EQA

<table>
<thead>
<tr>
<th>Test</th>
<th>EGFR IHC</th>
<th>EGFR ISH</th>
<th>EGFR mutation</th>
<th>KRAS Mutation</th>
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<tbody>
<tr>
<td>Consensus</td>
<td>4/17</td>
<td>17/17</td>
<td>13/13</td>
<td>5/5</td>
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<tr>
<td>Labs</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>9</td>
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<tr>
<td>NA cases</td>
<td>15 / 136</td>
<td>3 / 117</td>
<td>1 / 45</td>
<td>2 / 153</td>
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<tr>
<td>Success rate</td>
<td>-</td>
<td>89±27%(^1)</td>
<td>97±4%</td>
<td>98±7%</td>
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<tr>
<td>Positive cases</td>
<td>-</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>FN</td>
<td>-</td>
<td>3 / 22</td>
<td>0 / 27</td>
<td>0 / 18</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>-</td>
<td>88±35%(^3)</td>
<td>100±0%</td>
<td>100±0%</td>
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<tr>
<td>Negative cases</td>
<td>-</td>
<td>14</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>FP</td>
<td>-</td>
<td>1 / 99</td>
<td>0 / 87</td>
<td>0 / 26</td>
</tr>
<tr>
<td>Specificity</td>
<td>-</td>
<td>96±12%(^5)</td>
<td>100±0%</td>
<td>100±0%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>-</td>
<td>95±10%(^6)</td>
<td>100±0%</td>
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<tr>
<td>TMA</td>
<td>ISH 2008</td>
<td>EGFR 2009</td>
<td>KRAS 2009</td>
<td>A</td>
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<td>Mut EGFR</td>
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<td>13</td>
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</tbody>
</table>

- **Mutation analysis TMA 2009**
- **Consensus**
- **Wrong low% T cells**
- **FP/ FN**

Thunnissen, 2011 JCP
Estimation of % of tumor cells for each TMA sample

Website calibration for estimation % tumor cells available ~2011
EGFR pilot

• Simon Patton, EMQN
• ETOP sponsored by Astra Zeneca, Roche

• 24 labs (10ESP Krakow, other connections)
EGFR pilot

- PCR sequencing n=8 (18-21 n=7, 18,19,21 n=1)
- DXS old n=8, new n=2
- HRM pyroseq. n=3, seq n=1
- Taqman 858 fragment length del 19 n=2
Score per case

- False positive/ negative 0
- No result/ failure to amplify 50%
- One mutation missing 50%
- Error in genotyping or protein typing 0.75%
- Ok = 100%*)

*) FOR THE FRAGMNENTS TESTED
EGFR mutation score per sample

- 1 79% FN 4,
- 2 95%
- 3 79% FP 5
- 4 77% FP 5
- 5 78% FP 5
- 6 79% FN 5
- 7 93% FN2
- 8 98%
- 9 98%
- 10 98%
EGFR mutation score per sample

- Each sample contained sample number plus block number
- 5 samples >95% most the same result
- 5 samples 77-79%!!

- Changed sample # for block #,
- (reverse: frequently correct outcome): sample registration error 5 labs same mistake
Score per lab

• 10 /10  n = 14
• 9,5 /10  n =  3
•  9 /10  n =  2
•  4-5 /10 n =  5
Interim conclusion

• Handling/ registration issue needs attention 5/24 labs (20%)

• At analytical level well performed 2 FN (0.8%)

• Feed back on reporting at individual level
ESP Proposal European QC EGFR testing

krakow:

ESP/ EMQN
Minimum set of (10) samples

National
-optional
additional samples

National
-optional
additional samples

National
-optional
additional samples

National
-optional
additional samples
Questions?