A New Gold Standard for Detection of Theranostic Gene Rearrangements and MET exon 14 splicing in Thoracic Oncology is Born!

One-Year Prospective Routine LD-RTPCR in 413 Newly Diagnosed Lung Tumors

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Background

Background

Background
- Cost
- Interpretation
- No identification of the partner gene
Ligation-dependent RT-PCR: a new specific and low-cost technique to detect ALK, ROS, and RET rearrangements in lung adenocarcinoma

Nicolas Piton\textsuperscript{1,2}, Philippe Ruminy\textsuperscript{2}, Claire Gravet\textsuperscript{1}, Vinciane Marchand\textsuperscript{2}, Élodie Colasse\textsuperscript{1}, Aude Lamy\textsuperscript{1}, Cécile Le Naoures Mear\textsuperscript{3}, Frédéric Bibeau\textsuperscript{3}, Florent Marguet\textsuperscript{1}, Florian Guisier\textsuperscript{4}, Mathieu Salaün\textsuperscript{4}, Luc Thiberville\textsuperscript{4}, Fabrice Jardin\textsuperscript{3} and Jean-Christophe Sabourin\textsuperscript{1,2}

Background

Background

Background

Retrospective study on:

- 24 ALK translocated

- 14 ROS translocated and

- 1 RET translocated tumors

### Background

<table>
<thead>
<tr>
<th>Tumors $ALK^+$ (n = 24)</th>
<th>Sensitivity: 63%</th>
<th>Specificity: 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumors $ROS^+$ (n = 14)</td>
<td>Sensitivity: 64%</td>
<td>Specificity: 100%</td>
</tr>
<tr>
<td>Tumors $RET^+$ (n = 1)</td>
<td>Sensitivity: 100%</td>
<td>Specificity: 100%</td>
</tr>
</tbody>
</table>

To assess an upgraded version of this assay in a routine **prospective** cohort of lung carcinomas for **one year**

**Improvements:**

- More probes
- High throughput sequencing
- Detection of *MET* exon 14 skipping
Material and methods

FFPE BLOCK OF DIAGNOSED LUNG CANCER

- GENOTYPING
- ALK IHC
- ROS IHC
- ALK FISH
- ROS FISH

IF POSITIVE

FURTHER TECHNIQUES

LD-RTPCR

IF DISCORDANCE WITH STANDARD PROCESS RESULTS

STANDARD PROCESS
Material and methods

47 probes

- EML4
- KLC1
- HIP1
- KIF5B
- STRN
- SEC31A
- TPR
- BIRC6
- DCTN1
- SQSTM1
- CLTC
- MSN
- CD74
- SLC34A2
- EZR
- SDC4
- LRIG3
- TPM3
- GOPC
- CCDC6
- NCOA4
- TRIM33

ALK

ROS

RET
Material and methods
Material and methods

Sequencing

FlowCell Illumina

Gene A    Gene B

Material and methods
Material and methods

RNA Patient 1 → RT 1 → Hyb 1 → PCR 1 → Purif 1 → Index 1

RNA Patient 2 → RT 2 → Hyb 2 → PCR 2 → Purif 2 → Index 2

RNA Patient 3 → RT 3 → Hyb 3 → PCR 3 → Purif 3 → Index 3

Pool

FlowCell « Micro »

Up to 40 patients (100,000 reads / Pt)
Material and methods

Exon 13 to Exon 15

No MET exon 14 splicing

52 bp

49 bp

55 bp

53 bp

Exon 13

Exon 14

Exon 15

MET exon 14 splicing

52 pb

*Wild-type MET

Exon 13

Exon 14

Exon 15

PCR length

Exon A

Exon B
# Results and discussion

<table>
<thead>
<tr>
<th></th>
<th>LD-RTPCR</th>
<th>Routine analysis</th>
</tr>
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<tbody>
<tr>
<td><strong>ALK translocation</strong></td>
<td>0</td>
<td>15 3</td>
</tr>
<tr>
<td><strong>ROS translocation</strong></td>
<td>0</td>
<td>4 0</td>
</tr>
<tr>
<td><strong>RET translocation</strong></td>
<td>2</td>
<td>0 0</td>
</tr>
<tr>
<td><strong>MET exon 14 splicing</strong></td>
<td>5</td>
<td>6 0</td>
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</table>

All molecular alterations above
Results and discussion

- 1 year prospective study

- 413 newly diagnosed lung tumors
## Results and discussion

### Routine analysis

<table>
<thead>
<tr>
<th>Routine techniques</th>
<th>ALK, ROS or RET translocation / MET exon 14 splicing</th>
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<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>28</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
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<tr>
<td>Total</td>
<td>35</td>
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</table>

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<th>95% CI</th>
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<tr>
<td><strong>Se</strong> = 80.00%</td>
</tr>
<tr>
<td><strong>Sp</strong> = 100.00%</td>
</tr>
<tr>
<td><strong>PPV</strong> = 100.00%</td>
</tr>
<tr>
<td><strong>NPV</strong> = 98.18%</td>
</tr>
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<td><strong>Accuracy</strong> = 98.31%</td>
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## Results and discussion

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</tr>
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<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>32</td>
<td>0</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>3</td>
<td>378</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>35</td>
<td>378</td>
<td>413</td>
</tr>
</tbody>
</table>

95% CI

- **Se =** 91.43%  
  76.94% to 98.20%
- **Sp =** 100.00%  
  99.03% to 100.00%
- **PPV =** 100.00%  
  97.59% to 99.72%
- **NPV =** 99.21%  
  97.71% to 99.73%
- **Accuracy =** 99.27%  
  97.89% to 99.85%
Results and discussion

- Cheap (< 10 € / test)
- Very fast (results < 1 week)
- Multiplex analyses
- 100% specific
- > 90% sensitivity
- Easily upgradable (e.g. NTRK and NRG1 genes)
Results and discussion

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A new technique of reference
Results and discussion

Non small cell lung carcinoma

IHC ALK ROS

Genotyping

LD-RTPCR

only if discordance

FISH
The authors would like to thank Dr Clotilde Descarpentries (Lille University Hospital) for genotyping some discordant cases and Pr Frédéric Bibeau (Caen University Hospital) for performing RET FISH.

Thanks for your attention!