Molecular pathology practice in Europe: how we do it

JC Sabourin
Department of Pathology
INSERM U1245
Rouen University Hospital
Normandy University
France
Introduction: clinical case

• Patient: male, born in 1954
• Never smoker
• Cough, chronic chest pain

Nodule located in the right inferior lobe on CT Scan

Endoscopy: nodule with neoplastic aspect

Biopsies +++
Diagnosis: Poorly Differentiated Adenocarcinoma, invasive, Probably of pulmonary origin (TTF1+, P40-)

Specimen (FFPE) referred to the molecular department
Molecular pathology results

**EGFR gene, exon 19: 12 bp deletion**

**ALK-, ROS-, KRAS-, HER2-, BRAF-**

Gefitinib (Iressa®) treatment for 10 months

New tumor progression (cerebral metastasis)

**IRESSA® treatment stopped**
Chemotherapy given

New Broncho-Endoscopy + biopsies
Diagnosis: Poorly differentiated adenocarcinoma (TTF1+)

- New EGFR genotyping in the molecular pathology department

  **EGFR gene, exon 19: 12 bp deletion**
  
  No resistance mutation to TKI

  Iressa® re-introduced

  New tumoral progression
  
  Third endoscopy + biopsies
Diagnosis: Small cell lung carcinoma
(SCLC, neuro-endocrine)

Same EGFR mutation

EGFR gene, exon 19: 12 bp deletion
EGFR-mutant adenocarcinomas, arising from type II alveolar cells, have the potential to become SCLC.

Analyses of repeat biopsy samples from patients with EGFR-mutant adenocarcinomas that transform to SCLC revealed that 100% of these patients have loss of RB1.

Loss of RB1 seems to be a universal event leading to transformation from adenocarcinoma to SCLC.
What type of specimen is used for these tests?

FFPE +++

Paraffin Blocks

5µm wide cutting (10µm for molecular biology)

- HE
- IHC
- FISH
- Mol Biology
On every lung biopsy/specimen: IHC: **TTF1, P40, PD-L1**

- Squamous K
- ADK

Molecular testing for targeted therapies

- IHC ALK and ROS, if +

FISH and LD-RT-PCR

- Fusion transcript testing
  - ALK, ROS1
  - RET, MET...

SNaPshot EGFR-KRAS

NGS
- 26 genes
- Illumina®

September 2019: Lung Testing
### Main mutation hotspots of numerous solid tumors

<table>
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<tr>
<th>Gene</th>
<th>Exons</th>
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<tbody>
<tr>
<td>AKT</td>
<td>exon 3</td>
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<td>PIK3CA</td>
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<tr>
<td>STK11</td>
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</table>

**Listes de gènes minimales à analyser dans le cadre d’un usage à visée diagnostique du NGS (février 2016)**

**Tumor Hotspot MASTR Plus**

**Agilent**
Liquid Biopsy Concept: From blood to other fluids...

Blood, Urine, Fluids...
EGFR mutation screening from blood samples!

Gefitinib Treatment in EGFR Mutated Caucasian NSCLC
Circulating-Free Tumor DNA as a Surrogate for Determination of EGFR Status

Jean-Yves Douillard, MD, PhD,* Gyula Ostoros, MD,† Manuel Cobo, MD,‡ Tudor Ciuleanu, MD,§ Rebecca Cole, PhD,‖ Gael McWalter, MSci,‖ Jill Walker, PhD,‖ Simon Dearden, MSc,‖ Alan Webster, MSc,‖ Tsveta Milenkova, MD,‖ and Rose McCormack, PhD‖

EMA was modified in the summer of 2014: if EGFR genotyping is not feasible on a tissue specimen, it is possible to search for EGFR mutation on a blood sample

Molecular Pathology is a new evolving branch of pathology, and it is now mandatory in specific indications to perform MP in order to offer the best possible diagnosis to our patients.

Molecular biology has long been used in haematopathology, but its integration in current practice for solid tumour pathology is more recent.

The development of targeted therapies has revolutionized pathology, with the need to know the mutation status of solid tumours (KRAS, EGFR, ALK etc.).

The introduction of high throughput techniques such as NGS was a new step in the revolution of our discipline.

And the new agnostic treatments such as NTRK inhibitors, will induce more and more tumour molecular screening.
Evolution of Molecular Pathology in Europe

• In 2016, the European Society of Pathology performed a survey « to give an overview of the practice of diagnostic molecular pathology in Europe »

• 56 questions to be answered

• The answers were presented at the ECP 2017 in Amsterdam
General information

**Is diagnostic molecular pathology performed routinely in your country?**

- Yes: 25
- No: 5

**Are cases routinely sent abroad for molecular testing?**

- Yes: 20
- No: 15

ESP 2016-2017 Survey
The following analyses are routinely performed in colorectal cancer:

- EGFR
- K-RAS
- B-RAF
- microsatellite instability (MSI)

Predictive mutational analysis of ovarian cancer (BRCA1/2) is routinely performed.

The following analyses are routinely performed in non-small cell lung cancer:

- EGFR
- K-RAS
- B-RAF
- others
Techniques used for diagnostic molecular testing

The following techniques are routinely available for molecular testing:

- **PCR-based techniques (allele-specific PCR, RFLP, melting curve analysis etc.)**
  - At my institution: 25
  - At another institution: 5
  - Not available: 0

- **Quantitative PCR techniques (e.g. TaqMan etc.)**
  - At my institution: 18
  - At another institution: 10
  - Not available: 0

- **Sanger sequencing**
  - At my institution: 16
  - At another institution: 12
  - Not available: 0

- **Next generation sequencing (NGS)**
  - At my institution: 16
  - At another institution: 12
  - Not available: 0
Professional and institutional affiliation of diagnostic molecular pathology

Which specialty is in charge of performing molecular testing in solid tumors? (multiple answers are possible)

Which specialty is in charge of signing out molecular testing in solid tumors? (multiple answers are possible)

At which types of institutions is molecular testing performed in your country? (multiple answers are possible)
Quality control in diagnostic molecular pathology

Accreditation

- Is accreditation or certification for diagnostic molecular pathology available in your country?
- Accreditation, is it mandatory?

EQA

- Is there a national (or other) external quality control assessment (EQA) for molecular diagnostic pathology in place in your country?
- Is EQA participation mandatory?
Reimbursement in diagnostic molecular pathology

The cost of diagnostic molecular pathology in general is covered by national health system/universal health insurance.

Which of the following common tests are covered by national health system/universal health insurance?

- HER2 FISH (breast)
- K/NRAS (colon)
- EGFR (lung)
In this symposium, a panel of European pathologists will address the following questions:

• Who performs MP?
• How is MP done? (Sanger, NGS, ready to use/automatic devices)
• How is MP organised in each country?
• Is MP covered by the national social security system/private medical insurance?
• How much does an EGFR and an NGS (< 20 kb) test cost?
• Are MP results part of the pathology report?
• What about accreditation according to ISO 15189 standard?
• How are pathologists trained for MP?
With examples in each country:

- How many MP tests are performed in a year?
- Description of these tests
- Is the Ministry of Health involved in the promotion/development of MP?
- What about collaboration with commercial solutions as Foundation Medicine, Myriad Genetics etc.
- What is the involvement of the pharmaceutical industry?
- Are Liquid Biopsies part of MP practice?
Who performs molecular pathology?
Romania: Pathology/Genetics Departments
Hungary: Pathology department & mol path lab

If it is a pathologist (MD), how is she/he trained? is there a special degree / diploma?
Romania: No special exam required
Hungary: Special exam in molecular pathology

Are molecular pathology acts reimbursed by your social security system?
Romania: No
Hungary: Partially reimbursed

Is all the population covered by the molecular pathology network?
Romania: No
Hungary: Yes

Source: Dr Sergiu SUSMAN MD PhD, Cluj-Napoca, Romania
How is molecular pathology organised: local centres? centralisation? reference centers?

Romania: Reference Centers

Hungary: Reference Centers

Molecular Pathology reports: are they standardized? are they part of the pathology report or on a separate report?

Romania: Separate standardized report

Hungary: Separate standardized report

Do you need ISO 15189 accreditation? (or other quality control regulations)

Romania: Yes

Hungary: Yes
Roughly, how many molecular pathology tests are performed in your country per year?

Romania: No available data
Hungary: For solid tumors approximately 9000

Is NGS performed in the pathology department or in a centralized platform (hospital/regional)? with which devices?

Romania: IonTorrent & Illumina
Hungary: IonTorrent & Illumina

Does the Ministry of Health promote/fund molecular pathology?

Romania: Yes, at least it tries
Hungary: Yes
Are there national/regional databases in order to compare molecular pathology results with the clinical outcomes of patients

Romania: Not for now, in the near future  
Hungary: Not yet, pending

What is pharmaceutical company involvement?

Romania: Important  
Hungary: Minimal

Do you perform Liquid Biopsies? Which biomarkers do you screen with Liquid biopsy?

Romania: Yes, *EGFR*  
Hungary: Yes *RAS, EGFR, ALK*
Central and Eastern Europe:
Austria, Bulgaria, Croatia, Czech Republic, Hungary, Poland, Romania, Serbia, Slovakia, Slovenia,
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<thead>
<tr>
<th>Country</th>
<th>EGFR testing</th>
<th>ALK testing</th>
<th>ROS1</th>
<th>PD-L1</th>
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Abbreviations: EGFR, epidermal growth factor receptor; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry; NA, not applicable; NSCLC-NOS, non-small cell lung cancer—not otherwise specified; PD-L1, programmed death-ligand 1.
Table 3. Availability and reimbursement of testing for EGFR mutation, ALK rearrangement, ROS1 mutation, BRAF mutation, and PD-L1 overexpression and of liquid biopsies in countries of central and southeastern Europe

<table>
<thead>
<tr>
<th>Country</th>
<th>EGFR (NSCLC) Test</th>
<th>Reimbursement</th>
<th>ALK Test</th>
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Abbreviations: EGFR, epidermal growth factor receptor; NA, not applicable; NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand 1.
Dr Falko Fend
Institute of Pathology and Neuropathology
Dept. of General and Molecular Pathology and Pathological Anatomy
Tübingen University Hospital
Tübingen, Germany

Dr Karin Oien
Institute of Cancer Sciences,
College of Medical Veterinary and Life Sciences, University of Glasgow, United Kingdom

Dr Carlo Della Rocca
Department of Medico-Surgical Sciences and Biotechnologies
Faculty of Pharmacy and Medicine “Sapienza” University of Rome, Italy

Dr Enrique de Alava
Pathology department
Virgen del Rocío University Hospital,
University of Seville, Seville
Spain

Dr Anders Edsjö
Department of Clinical Genetics and Pathology,
Division of Laboratory Medicine, Office for Medical Services,
Region Skåne, Malmö, Sweden

Dr Jean-Christophe B Sabourin
Department of Pathology Biology Division
Rouen University Hospital
Normandy University,
France
A new survey on MP practice in Europe will be launched by the ESP in a few weeks and the results will be published in Virchows Archiv.
Molecular Pathology in nice in NICE!

Thank you for your attention