DIAGNOSTIC WORK UP IN NSCLC AND
THE IMPORTANCE OF OPTIMAL
TISSUE MANAGEMENT IN THE ERA OF
PRECISION MEDICINE

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Advanced NSCLC: a need for personalised therapy

Diagnostic workup of lung cancer:
- Type of samples
- Morphology and histology
- Material for biomarker studies

Overview of key genetic alterations in NSCLC
- Techniques for diagnose

Guidelines for genetic testing

Summary / conclusions
OUTLINE

◆ Advanced NSCLC: a need for personalised therapy
◆ Diagnostic workup of lung cancer:
  □ Type of samples
  □ Morphology and histology
  □ Material for biomarker studies
◆ Overview of key genetic alterations in NSCLC
  □ Techniques for diagnose
◆ Guidelines for genetic testing
◆ Summary / conclusions
EVOLUTION OF LUNG CANCER DIAGNOSIS

Lung cancer classification has moved from histologic to molecular classification.

FISH, fluorescent in situ hybridisation; H&E, hematoxylin and eosin stain; IHC, immunohistochemistry.

Images courtesy of Dr C Teixidó and Dr N Reguart
NSCLC MOLECULAR CLASSIFICATION

NSCLC classification has moved from histologic to molecular subtyping.

Molecular Testing: Adenocarcinoma and NSCLC-NOS histologies

Adapted from Li T, et al. J Clin Oncol 2013;31:1039–49

NOS, not otherwise specified.
### Driver ONCOGENES IN LUNG ADENOCARCINOMA BY ETHNICITY

<table>
<thead>
<tr>
<th>Gene</th>
<th>USA/Europe (%)</th>
<th>East Asia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EGFR</strong></td>
<td>5-15%</td>
<td>40-55%</td>
</tr>
<tr>
<td><strong>KRAS</strong></td>
<td>20-30%</td>
<td>8-10%</td>
</tr>
<tr>
<td><strong>ALK</strong></td>
<td>3-6%</td>
<td>3-5%</td>
</tr>
<tr>
<td><strong>ROS1</strong></td>
<td>1-2%</td>
<td>2-3%</td>
</tr>
<tr>
<td><strong>BRAF</strong></td>
<td>2-3%</td>
<td>0.5-1%</td>
</tr>
<tr>
<td><strong>RET</strong></td>
<td>1-2%</td>
<td>1-2%</td>
</tr>
<tr>
<td><strong>MET</strong></td>
<td>4%</td>
<td>1.3%</td>
</tr>
<tr>
<td><strong>HER2</strong></td>
<td>2-3%</td>
<td>2-3%</td>
</tr>
<tr>
<td><strong>NTRK</strong></td>
<td>1%</td>
<td>&lt;1%</td>
</tr>
</tbody>
</table>

More than 50% of all lung adenocarcinomas harbour driver oncogenes. Incidence of genomic driver is variable among ethnic populations.

References:
METASTATIC NSCLC: ESMO CLINICAL PRACTICE GUIDELINES

Stage IV Non-Squamous Cell Carcinoma

Molecular Tests Positive (ALK/BRAF/EGFR/ROS1)

Molecular Tests Negative (PD-L1 expression)

Stage IV Squamous Cell Carcinoma

LoE Levels of evidence (I-V); GoR nd grades of recommendation (A-E); TMB, tumour mutational burden.

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- Guidelines for genetic testing
- Summary / conclusions
Before starting, review relevant clinical information, such as:
- Sample site
- Clinical suspicion on primary lung tumour or metastasis
- Reason for biopsy: diagnosis, molecular evaluation, resistance
- Smoking history

Then, establish diagnostic work up:
- **FIRST** → Establish malignancy
- **SECOND** → Define type of malignancy
- **THIRD** → Perform molecular testing as appropriate and prioritise testing according to individual patients
Diagnostic material is generally scarce and it is essential to balance requirements for an accurate histologic diagnosis with the need for molecular analyses.

When biopsy and cytology material from an individual patient are analysed by different professional (histopathologists and cytologists), it is essential that they share information.

40% NSCLC diagnosed by cytology.
HISTOLOGY: IMMUNOHISTOCHEMICAL MARKERS

Type of malignancy

<table>
<thead>
<tr>
<th>TUMOUR TYPE</th>
<th>IHC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma (ADC)</td>
<td>CK7, TTF-1</td>
</tr>
<tr>
<td>Squamous cell carcinoma (SCC)</td>
<td>P40 (P63, less specific)</td>
</tr>
<tr>
<td>Neuroendocrine (LCNEC, SCLC)</td>
<td>CD56 (NSE, chromogranin, synaptophysin)</td>
</tr>
</tbody>
</table>

1. IHC should not be performed unless necessary!!!!
2. In NSCLC-NOS, IHC reduces the number of inconclusive diagnoses (rate <10%)
3. Limiting IHC between 2 and 4 markers seems reasonable

In NSCLC biopsy samples the following terminology should be used:

- ADC IHC not required if diagnostic morphology present
- SCC IHC not required if diagnostic morphology present
- Carcinomas lacking clear differentiation by morphology and special stains are classified as NSCLC-NOS
- NOS that stain with adenocarcinoma markers, classified as NSCLC, favor adenocarcinoma
- NOS that stain with squamous markers, classified as NSCLC, favor squamous cell carcinoma

ADC, adenocarcinoma; LCNEC, large cell neuroendocrine carcinoma; NOS not otherwise specified; NSCLC, non-small cell lung cancer; SCC, squamous cell carcinoma; SCLC, small-cell lung cancer.
HISTOLOGY: IMMUNOHISTOCHEMICAL MARKERS

Type of malignancy

Lung adenocarcinoma, TTF1 positive

Squamous cell carcinoma, P40 positive

Images courtesy of Dr D. Martinez, Hospital Clinic Barcelona
TISSUE QUALITY CONTROL FOR MOLECULAR TESTING

Pathologist review H&E section and mark areas for analysis

The molecular specialist must evaluate whether the percentage of tumour in a given specimen reaches the detection threshold of the specific molecular test or tests.

Assess tumour percentage and viability
Select ADC if mixed for testing

Images courtesy of Dr C Teixidó and Dr N Reguart

ADC, adenocarcinoma; H&E, haematoxylin and eosin stain; T, tumour.
MOLECULAR TECHNIQUES AVAILABLE IN ROUTINE DIAGNOSIS

**Single-marker molecular tests**
- PCR
- FISH
- IHC

**Multiplex-marker molecular tests**
- Multiplex test
- Massively parallel / NGS

FISH, fluorescent in situ hybridisation; IHC, immunohistochemistry; NGS, next generation sequencing; PCR, polymerase chain reaction.

Images courtesy of Dr C Teixidó and Dr N Reguart
MOLECULAR TECHNIQUES AVAILABLE IN ROUTINE DIAGNOSIS

Tissue requirements

- **H&E SAMPLE**
  - Whole tissue sections
  - Selection on tumour areas
  - ~50-100 cells*

- **H&E SAMPLE**
  - IHC
  - FISH
  - ~50-100 cells*

- **H&E SAMPLE**
  - RT-PCR
  - nCounter
  - NGS
  - ~600-1000 cells*

*Numbers are approximations and tissue requirements depends on technique/reagent/platform

FISH, fluorescent in situ hybridisation; H&E, hematoxylin and eosin stain; IHC, immunohistochemistry; NGS, next generation sequencing; RT-PCR, reverse transcription polymerase chain reaction

Images courtesy of Dr C Teixidó and Dr N Reguart
STRATEGY TO MAXIMISE TISSUE FOR MOLECULAR TESTING

At the time of initial sectioning for morphologic diagnosis, additional sections may be taken, in anticipation of IHC or predictive markers analyses.

- Technicians should preserve as much tissue as possible for further evaluations.

Using cytology samples, cell block preparation from clot formed by remaining fluid should be obtained in combination with smears to improve diagnostic information.

Molecular testing sections can be prepared:
- Together with first H&E section
- After initial H&E for histological diagnosis

H&E, haematoxylin and eosin stain; IHC, immunohistochemistry

Images courtesy of Dr C Teixidó and Dr N Reguart
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EGFR
Epidermal Growth Factor Receptor
HOTSPOTS FOR EGFR GENE MUTATIONS

◆ Activating (and sensitising) EGFR mutations are predictive for response to EGFR tyrosine kinase inhibitors

◆ More common:
  - Never-smokers
  - Women
  - Adenocarcinoma subtype

◆ Alterations:
  - Insertions
  - Deletions
  - Point mutations

**EGFR MUTATION ASSAYS**

CAP/IASLC/AMP recommended assays for EGFR genotyping testing

### Non-squamous-cell NSCLC
- Adenocarcinoma
- Large-cell carcinoma
- Other

### PCR-based methods

- Sanger direct sequencing: 25%
- Real time/ TaqMan PCR: 10%
- High Resolution melting analysis: 5-10%
- Cobas: 5-10%
- Pyrosequencing: 5-10%
- SNaPshot PCR: 5-10%
- MALDI-TOF MS-based genotyping: 5%
- Cycleave PCR: 5%
- Fragment lenght and RFLP analysis: 5%
- Allelic specific PCR/ Scorpion ARMS: 1%
- MassARRAY: 1%
- PNA- LNA PCR clamp: 1%
- Denaturing HPLC: 1%
- Massively parallel/ NGS: 0.1%

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**NOTE:** AMP, Association for Molecular Pathology; ARMS, amplification refractory mutation system; CAP, College of American Pathologists; HPLC, high performance liquid chromatography; IASLC, International Association for the Study of Lung Cancer; LNA, locked nucleic acid; NGS, next generation sequencing; PCR, polymerase chain reaction; PNA, peptide nucleic acid; RFLP, restriction fragment length polymorphism

Adapted from IASLC Textbook in Thoracic Oncology 2014

**EGFR TESTING IN CELL-FREE PLASMA DNA**

The dynamic nature of EGFR resistance mechanisms can be monitored in cfDNA

- **Insufficient evidence** to support the use of cfDNA for the diagnosis of primary lung adenocarcinoma

- When tissue is **limited** and/or insufficient for molecular testing, physicians may use a cfDNA assay to identify EGFR mutations as an alternative

- Physicians may use cfDNA methods to identify **EGFR T790M** mutations in lung adenocarcinoma patients with progression or secondary clinical resistance to EGFR-targeted TKI

- Testing of the tumour sample is recommended if cfDNA result is negative

cfDNA, cell-free DNA; TKI, tyrosine-kinase inhibitor
Planchard D. et al. Ann Oncol 2018;29(Supplement_4):iv192-iv237, by permission of Oxford University Press on behalf of the European Society for Medical Oncology
ALK
Anaplastic Lymphoma Kinase
**ALK FUSION GENE**

- **ALK** fusion gene results in formation of cytoplasmic chimeric proteins with constitutive kinase activity
- *The ALK* fusion gene appears to be a distinct NSCLC molecular subset susceptible to targeted inhibition
- Tends to be mutually exclusive with *EGFR* and *KRAS* mutations
- More common:
  - Never or light smokers
  - Younger age
  - Adenocarcinoma subtype

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*No specific page references are provided in the text.*
**ALK FUSION GENE**

- **Most common partner** *EML4* (90%)
- Produces oncogenic *EML4-ALK*-fusion protein
- Protein activation by partner-provided **coiled-coil dimerisation domain**
- Breakpoint within *ALK* always at **exon 20**
- Breakpoint within *EML4* differ: **21 variants**

**Breakpoints within EML4**

<table>
<thead>
<tr>
<th>Breakpoints</th>
<th>2</th>
<th>6</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>17</th>
<th>18</th>
<th>20</th>
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<tbody>
<tr>
<td>EML4</td>
<td></td>
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</tbody>
</table>

**Variants of the -fusion protein**

<table>
<thead>
<tr>
<th>Fusion protein</th>
<th>Variant</th>
<th>Frequency in ALK+ NSCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>EML4</td>
<td>ALK</td>
<td>E13;A20 (V1) 33%</td>
</tr>
<tr>
<td>EML4</td>
<td>ALK</td>
<td>E6a/b;A20 (V3a/b) 29%</td>
</tr>
<tr>
<td>EML4</td>
<td>ALK</td>
<td>E20;A20 (V2) 9%</td>
</tr>
<tr>
<td>EML4</td>
<td>ALK</td>
<td>E14;A20 (V4) 3%</td>
</tr>
<tr>
<td>EML4</td>
<td>ALK</td>
<td>E18;A20 (V5) 2%</td>
</tr>
<tr>
<td>EML4</td>
<td>ALK</td>
<td>E14;A20 (V7) 2%</td>
</tr>
<tr>
<td>EML4</td>
<td>ALK</td>
<td>E2a/b:A20 (V5a/b) 2%</td>
</tr>
<tr>
<td>EML4</td>
<td>ALK</td>
<td>E17;A20 (V8) 1%</td>
</tr>
<tr>
<td>KIF5B</td>
<td>ALK</td>
<td></td>
</tr>
<tr>
<td>TFG</td>
<td>ALK</td>
<td></td>
</tr>
<tr>
<td>KLC1</td>
<td>ALK</td>
<td></td>
</tr>
<tr>
<td>PTPN3</td>
<td>ALK</td>
<td></td>
</tr>
<tr>
<td>STRN</td>
<td>ALK</td>
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</tbody>
</table>

**Breakpoints within *EML4***

TESTING FOR ALK GENE FUSION

ALK fusion variants in NSCLC

- The ALK break-apart FISH assay detects ALK fusions in FFPE NSCLC tissue specimens
- Considered ALK positive if >15% cell with break-apart
- The centromeric (green) and telomeric (red) probes flank the ALK locus
  - Splitting probes of the red and green signals indicates ALK fusion
  - A yellow signal indicates no ALK fusion
- ALK IHC (D5F3, Ventana or 5A4, Novocastra) may be used as a screening test

<table>
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</table>
TESTING FOR ALK GENE FUSION

Diagnostic algorithm that uses IHC as the primary test for ALK identification

- Considered ALK IHC positive if strong granular cytoplasmic staining with/without membrane accentuation
- Occasional cases may be difficult to interpret because of heterogeneous fixation/preservation and/or nonspecific staining artefacts (e.g., staining in alveolar macrophages, neural cells, extracellular mucin, necrosis). In these settings, these cases should also be tested by a validated method (e.g., ALK FISH)

FFPE, formalin-fixed paraffin embedded; FISH, fluorescent in situ hybridisation; IHC, immunohistochemistry
TESTING FOR ALK GENE FUSION

FISH BAP Menarini

IHC D5F3 Ventana

IHC 5A4 Novocastra

BAP, break-apart probe; FISH, fluorescent in situ hybridisation; IHC, immunohistochemistry
Images courtesy of Dr C Teixidó and Dr N Reguart
ROS1

c-ROS Oncogene 1
The ROS1 fusion gene appears to be a distinct NSCLC molecular subset susceptible to targeted inhibition.

Tends to be mutually exclusive with EGFR, KRAS, ALK alterations.

More common:
- Never or light smokers
- Younger age
- Adenocarcinoma subtype

The mechanism by which the ROS1 fusion protein is activated remains unclear.
**ROS1 FUSION GENE**

Many partners, most common **CD74 (30%)**

Produces oncogenic **ROS1 kinase protein**

**Unknown** mechanism of protein activation (no coiled-coil dimerisation domain provided)

Breakpoint sites vary **exons 32, 34, 35**

**Breakpoints within ROS1**

Breakpoints 32 34 35

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## TESTING FOR ROS1 GENE FUSION

<table>
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<tr>
<td>nCounter</td>
</tr>
<tr>
<td>Next Generation Sequencing (NGS)</td>
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</table>

- FISH is the trial-validated standard. The ROS1 FISH testing should be performed with a break-apart probe design given the multiple fusion partners described.

- Considered ROS1 positive if >15% cell with break-apart.

- ROS1 IHC (D4D6, Cell Signalling Technology) may be used as a screening test in lung adenocarcinoma patients; however, positive ROS1 IHC results should be confirmed by an orthogonal method - cytogenetic method (FISH) or molecular.

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FISH, fluorescent in situ hybridisation; IHC, immunohistochemistry
BRAF

B-raf Proto-Oncogene
Activating mutations in *BRAF*, especially p.V600E, lead to oncogenic signaling through MAPK

*BRAF V600E* mutations appear to be a distinct NSCLC molecular subset susceptible to targeted inhibition: *BRAF/MEK* inhibitors

Typically mutually exclusive of other oncogenic drivers

It is appropriate to include *BRAF* as either part of larger testing panels performed initially or when routine *EGFR*, *ALK*, and *ROS1* testing are negative

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HOTSPOTS FOR *BRAF* GENE MUTATIONS

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From files of Teixidó C, Reguart N

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*N*, N-terminal; *C*, C-terminal; RBD, RAS-binding domain; PL, P-loop; αC, alpha C hélix motif; DF, dimerization interface; CL, catalytic loop; AS, activation site; aa, amino acid
RET
REarranged during Transfection
**RET FUSION GENE**

Not currently indicated as routine stand-alone test outside the context of a clinical trial

- The *RET* fusion gene appears to be a distinct NSCLC molecular subset susceptible to targeted inhibition
- Tends to be mutually exclusive with other oncogenic drivers

- More common:
  - Never or light smokers
  - Younger age
  - Adenocarcinoma subtype

- To date, no *RET*-directed targeted therapeutic has received regulatory approval for *RET*-mutant or *RET*-rearranged solid tumours

RET FUSION GENE

- Six partners, most common **KIF5B (30%)**
- Produces oncogenic **RET kinase protein**
- Protein activation by partner-provided **coiled-coil dimerisation domain**
- Breakpoint sites vary **exons 11, 12**

**Breakpoints within RET**

Breakpoints 11 12

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**Takeuchi K, et al. Nat Med 2012; Figure republished with permission of Pioneer Bioscience Publishing Company, from Transl Lung Cancer Res, Kohno T, et al. 4(2), 2015; permission conveyed through Copyright Clearance Center, Inc.**
TESTING FOR RET GENE FUSION
Not currently indicated as routine stand-alone test outside the context of a clinical trial

**TECHNIQUES**

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<tr>
<th>Technique</th>
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<tbody>
<tr>
<td>Fluorescent in-situ hybridisation (FISH)</td>
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<tr>
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</tbody>
</table>

- The RET FISH testing should be performed with a break-apart probe design given the multiple fusion partners described.
- Considered RET positive if >15% cell with break-apart.
- RET fusion genes cannot be adequately detected by IHC.

IHC, immunohistochemistry; FISH, fluorescent in situ hybridisation


NTRK
Neurotrophic tyrosine kinase
**NTRK FUSION GENE**

Family of neurotrophin receptors (*NTRK* 1-3)

*NTRK* fusion gene appears to be a distinct NSCLC molecular subset susceptible to targeted inhibition

- Tends to be mutually exclusive with other oncogenic drivers

- Prevalence in unselected population
  - Occur across age and smoking status
  - No gender preference

- To date, no *NTRK*-directed targeted therapeutic has an approval for *NTRK*-gene fusion

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**NTRK FUSION GENE**

Neurotrophic receptors: **NTRK1, NTRK2, NTRK3**

Transmembrane proteins: **TRKA, TRKB and TRKC**

Most common fusion in lung **NTRK1**

Most common partners **CD74 and MPRIP**

Oncogenic constitutive activation of **TRKA**

**Unknown** mechanism of protein activation (no coiled-coil dimerisation domain provided)

Breakpoint sites vary **exons 12, 14**

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![Diagram](image.png)

**Breakpoints**
- **NTRK1**
- Exons 12 and 14

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# TESTING FOR NTRK GENE FUSION

## TECHNIQUES

<table>
<thead>
<tr>
<th>Method</th>
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<tr>
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</tbody>
</table>

- TRK protein testing can be considered as part of broad immunohistochemistry testing
- TRK IHC may be used as a screening test
- A TRK positive test should then be confirmed with NGS

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IHC, immunohistochemistry; NGS, next generation sequencing

MET

MET Proto-Oncogene
TESTING FOR MET GENE AMPLIFICATION

Not currently indicated as routine stand-alone test outside the context of a clinical trial

- FISH is the trial-standard technique but there is no guideline for cut-off of MET positivity
- High polysomy occurs when there are multiple copies of chromosome 7 (CEP7) in tumour cells (>5)
- MET amplification may be classified by using MET:CEP7 Ratio as low (≥1.8 to 2.2≤), intermediate (>2.2 to <5), and high (≥5)
- Low–intermediate levels can occur synchronously with other oncogenic mutations and gene rearrangements up to 63% of lung carcinomas

**TECHNIQUES**

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<td>Immunohistochemistry (IHC)</td>
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<td>Real time polymerase chain reaction (qRT-PCR)</td>
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<td>Next Generation Sequencing (NGS)</td>
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</tbody>
</table>

Image courtesy of Dr C Teixidó and Dr N Reguart

TESTING FOR MET EXON 14 SKIPPING MUTATIONS
Not currently indicated as routine stand-alone test outside the context of a clinical trial

Schematic illustration of some MET\(\Delta 14\)

◆ MET\(\Delta 14\) mutations exhibit a highly diverse sequence composition (insertions, deletions, SNV)

<table>
<thead>
<tr>
<th>TECHNIQUES</th>
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<tbody>
<tr>
<td>In-situ hybridisation (ISH)</td>
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<td>Reverse transcription polymerase chain reaction (RT-PCR)</td>
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<td>nCounter</td>
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</table>

SNV, single nucleotide variation
Reprinted from Cancer Discov 2015, 5(8):842–9, Paik PK, et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping, with permission from AACR.
PD-L1
Programmed Cell Death Ligand-1
PD-L1 TESTING FOR IMMUNOTHERAPY
Standard Detection by IHC

◆ PD-L1 expression has been detected on tumour cells and tumour-infiltrating immune cells

◆ PD-L1 on tumour cells may lead to the inhibition of activated T-cells

◆ To enrich for those patients more likely to benefit from anti-PD-1 or anti-PD-L1 therapy

◆ IHC to identify PD-L1 expression at the appropriate level and on the appropriate cell population(s) as determined by the intended drug and line of therapy
# PD-L1 IHC ASSAYS IN LUNG CANCER

Summary of PD-L1 antibodies and technical aspects for evaluation in NSCLC

<table>
<thead>
<tr>
<th>mAb clone</th>
<th>Ab host species</th>
<th>Platform</th>
<th>PD-L1 scoring</th>
<th>Cut-offs</th>
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<tbody>
<tr>
<td>22C3</td>
<td>Mouse</td>
<td>Dako</td>
<td>TC</td>
<td>TC ≥1% or TC ≥50%</td>
</tr>
<tr>
<td>28-8</td>
<td>Rabbit</td>
<td>Dako</td>
<td>TC</td>
<td>TC ≥1%</td>
</tr>
<tr>
<td>SP142</td>
<td>Rabbit</td>
<td>Ventana</td>
<td>TC, IC</td>
<td>TC ≥50% or IC ≥10%</td>
</tr>
<tr>
<td>SP263</td>
<td>Rabbit</td>
<td>Ventana</td>
<td>TC</td>
<td>TC ≥25%</td>
</tr>
<tr>
<td>73-10</td>
<td>Rabbit</td>
<td>Dako</td>
<td>TC</td>
<td>TC ≥1%</td>
</tr>
</tbody>
</table>

**Blueprint Phase 2a**

22C3, 28-8 and SP263 assays are comparable when used to determine PD-L1 status of patient’s tumour (TPS), SP142 detects less, while 73-10 stains more PD-L1 positive tumour cells.

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TC, tumour cells; IC, immune cells; TPS, tumour proportion score

Adapted from Teixidó C, et al. Ther Adv Med Oncol 2018;10:1–17

Tsao MS, et al. J Thorac Oncol. 2018 Sep;13(9):1302-1311
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  □ Material for biomarker studies
◆ Overview of key genetic alterations in NSCLC
  □ Techniques for diagnose
◆ Guidelines for genetic testing
◆ Summary / conclusions
Molecular pathology/biology recommendations

- Testing for EGFR mutation status [LoE, GoR: I, A], ALK rearrangement [I, A], ROS1 rearrangement [II, A], BRAF V600 mutation status [II, A] and PD-L1 expression by IHC [I, A] should be systematically analysed in advanced NSCLC.

- Molecular EGFR and ALK testing are not recommended in patients with a confident diagnosis of squamous, except in unusual cases, e.g., never/former light smokers or long-time ex-smokers [IV, A].
EGFR mutation testing should have adequate coverage of mutations in exons 18–21, including those associated with resistance to some therapies [LoE, GoR: III, B]

Detection of the ALK translocation by FISH remains a standard, but IHC with high-performance ALK antibodies and validated assays may be used for screening [III, A] and have recently been accepted as an equivalent alternative to FISH for ALK testing.

Detection of the ROS1 translocation by FISH remains a standard; IHC may be used as a screening approach [IV, A]

If available, multiplex platforms (NGS) for molecular testing are preferable [III, A]. Whatever testing modality is used, it is mandatory that adequate internal validation and quality control measures are in place and that laboratories participate in, and perform adequately in, external quality assurance schemes for each biomarker test [III, A]

FISH, fluorescent in situ hybridisation; GoR, grades of recommendation (A–E); IHC, immunohistochemistry; LoE, levels of evidence (I–V); NGS, next generation sequencing. Planchard D. et al. Ann Oncol 2018;29(Supplement_4):iv192-iv237
### METASTATIC NSCLC: ESMO CLINICAL PRACTICE

### Biomarker testing

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Method</th>
<th>Use</th>
<th>LoE, GoR</th>
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<tbody>
<tr>
<td><strong>EGFR mutation</strong></td>
<td>Any appropriate, validated method, subject to external quality assurance</td>
<td>To select those patients with <strong>EGFR</strong>-sensitising mutations most likely to respond to <strong>EGFR</strong> TKI therapy</td>
<td>I, A</td>
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<td><strong>ALK rearrangement</strong></td>
<td>Any appropriate, validated method, subject to external quality assurance. FISH is the historical standard but IHC is now becoming the primary therapy-determining test, provided the method is validated against FISH or some other orthogonal test approach. NGS is an emerging technology</td>
<td>To select those patients with <strong>ALK</strong> gene rearrangements most likely to respond to <strong>ALK</strong> TKI therapy</td>
<td>I, A</td>
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<tr>
<td><strong>ROS1 rearrangement</strong></td>
<td>FISH is the trial-validated standard. IHC may be used to select patients for confirmatory FISH testing but currently lacks specificity. NGS is an emerging technology. External quality assurance is essential</td>
<td>To select those patients with <strong>ROS1</strong> gene rearrangements most likely to respond to <strong>ROS1</strong> TKI therapy</td>
<td>II, A</td>
</tr>
<tr>
<td><strong>BRAF mutation</strong></td>
<td>Any appropriate, validated method, subject to external quality assurance</td>
<td>To select those patients with <strong>BRAF</strong> V600-sensitising mutations most likely to respond to <strong>BRAF</strong> inhibitor, with or without <strong>MEK</strong> inhibitor therapy</td>
<td>II, A</td>
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<tr>
<td><strong>PD-L1 expression</strong></td>
<td>IHC to identify PD-L1 expression at the appropriate level and on the appropriate cell population(s) as determined by the intended drug and line of therapy. Only specific trial assays are validated. Internal and external quality assurance are essential</td>
<td>To enrich for those patients more likely to benefit from anti-PD-1 or anti-PD-L1 therapy. For pembrolizumab, testing is a companion diagnostic for nivolumab and atezolizumab, testing is complementary</td>
<td>I, A</td>
</tr>
</tbody>
</table>

FISH, fluorescent *in situ* hybridisation; GoR, grades of recommendation (A–E); IHC, immunohistochemistry; LoE, levels of evidence (I–V); MEK, mitogen-activated protein kinase kinase; NGS, next generation sequencing; TKI, tyrosine-kinase inhibitor

SUMMARY / CONCLUSIONS

◆ In NSCLC, the determination of histologic subtype and molecular predictive markers are standard of care.

◆ Determination of EGFR, ALK, ROS1, BRAF and PD-L1 status in tumour specimens is currently standard of care in advanced NSCLC patients.

◆ In lung cancer, optimal management of biopsy specimens are needed to avoid repeat biopsies.
  ▪ Pathologists have a key role in treatment decisions.
  ▪ Closed interaction between pulmonologists, pathologists, biologists, and oncologists is required.

◆ In the era of personalised therapy, professionals involved in lung cancer diagnosis/management should develop their own multidisciplinary tissue management strategy to obtain specimens and process them.
THANK YOU!
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