ESMO ADVANCED COURSE ON BIOMARKERS FOR PRECISION MEDICINE:

Biomarkers for Immune Checkpoint Inhibitor Molecular Pathology

Paul Hofman, MD, PhD
Zürich, 28-29 November 2019

University Côte d’Azur and Inserm U1081 CNRS 7284, Nice, France
I herewith declare that, 

I have the following potential conflict(s) of interest to report:

Receipt of grants/research supports:
- Bristol-Myers Squibb

Receipt of honoraria or consultation fees:
- Bristol-Myers Squibb, Thermo Fisher Scientific, Qiagen, Illumina, AstraZeneca, Roche, AbbVie, Pierre Fabre, Boehringer Ingelheim, Novartis
ROAD MAP

Brief background in thoracic oncology

Which samples and which limitations?

Which biomarkers?

Which technology?

Take away message
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Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries

>2 million cases per year
>1.7 million deaths per year

Women/men
Aging
Pollutants

Changing in lung cancer landscape?
The main cancer killer

Aging and cancer
Two sides of the same coin

Ischemic heart disease
Cerebrovascular disease
Lower respiratory infection
Diarrheal disease
Perinatal disorders
COPD
Tuberculosis
Measles
Road traffic accidents

Lung cancer

Murray CJL, Lopez AD. Lancet; 349; 1269-76
First cause of death cancer in France (> 35,000 deaths in 2018)

Global increase of incidence in France in 2018
Current therapeutic strategies in lung cancer

An urgent need to offer a personalized treatment for all patients
First line treatment in late stage NSCLC

- Targeted therapy
- Immunotherapy
- Chemotherapy

and/or
First line treatment in late stage NSCLC (2018)

Challenge for precision medicine

Less chemotherapy, more personalized treatment
Some patients (\%) do not get the « good » treatment due to:

1) A long turnaround time for getting the results and/or,
2) A non appropriate testing (then receive chemo in first line)

An urgent need for optimizing the process
Some patients (%) do not receive the « good » treatment due to:

1) A long turnaround time for getting the results and/or,
2) A non appropriate testing (and receive chemo in first line)

An urgent need for optimizing the process

In 2018 only 72 % of french patients with late stage lung adenocarcinoma have been tested for EGFR
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Take away message
Surgical specimen

BAL/CSF/pleural fluids

Bronchial aspirates

Bronchial biopsy/Transthoracic Biopsy

Blood

EBUS
The right drug

The right patient

The right time

New therapies in lung cancer patients

The right approach

The right cost
The right turnaround time

The right sample

Stratification in cancer

The right patient

The right time

The right approach

The right cost
More to do from small sample

HE first
Diagnostic stain TTF1
Diagnostic stain mucin
Diagnostic stain p63/p40
Predictive stain ALK IHC
Predictive stain ROS1 IHC
Predictive stain PD-L1 IHC, etc
DNA isolation EGFR
DNA isolation
HE last

NGS
• miseq 40-250 ng
• Ion torrent 10 ng
Lung cancer and sampling management
Controversies on Lung Cancer: Pros and Cons

Pros: Can tissue biopsy be replaced by liquid biopsy?

Marius Ilié¹,²,³,⁴, Paul Hofman¹,²,³,⁴

Lung cancer and

Late stages NSCLC

1

Early stages NSCLC

2

Neoadjuvant immunotherapy

Next, coming soon

(see diagram for biopsy locations and follow-up visits)
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Take away message
Metastatic Non-Small-Cell Lung Cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up

Updated version published 18 September 2019 by the ESMO Guidelines Committee

Published 18 September 2019 by the ESMO Guidelines Committee


Download the current version from esmo.org (PDF)

Download the original version from Annals of Oncology (PDF)

CLINICAL PRACTICE GUIDELINES

Metastatic non-small cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†

D. Planchard¹, S. Popat², K. Kerr³, S. Novello⁴, E. F. Smit⁵, C. Faivre-Finn⁶, T. S. Mok⁷, M. Reck⁸, P. E. Van Schil⁹, M. D. Hellmann¹⁰ & S. Peters¹¹, on behalf of the ESMO Guidelines Committee*
For advanced or metastatic lung adenocarcinoma: It is mandatory that all patients need, 1) to have testing for EGFR and BRAF mutation, ALK and ROS1 rearrangements, and PD-L1 expression to predict response to EGFR, ALK, ROS1 or BRAF targeted inhibitors or immunotherapy, respectively, and, 2) to get results in ten working days.

*Other biomarkers are under investigation as predictors of response to targeted therapies, in particular Her2 mutation, RET and NTRK rearrangements and MET splice mutations and amplification.

For advanced or metastatic lung squamous carcinoma: PD-L1 IHC is indicated to select patients for immunotherapy in the first line.
Stages IIIB/IV non squamous cell carcinoma of the lung

10 working days

Mandatory

- PD-L1 IHC
- ALK IHC and/or ALK FISH
- ROS1 IHC then ROS1 FISH
- EGFR mutations
- BRAFV600 mutations

Recommended

- HER2 mutation
- NTRK rearrangement
- RET rearrangement
- MET mutation
- MET amplification

Large gene panel

Stages IIIB/IV squamous cell carcinoma of the lung

PD-L1 IHC
The **only validated test** as a predictive biomarker of I-O first line

- Patients with 0% of PD-L1 TC positivity can be good responders
- Patients with > 50% of PD-L1 TC positivity can be non responders
- Heterogeneity of PD-L1 staining limits its interpretation in small biopsies
- Inter & intra operator variability in PD-L1 IHC assessment
- Many PD-L1 clones, different devices, different performances
- Many cut off (>1%, > 25%, > 50%) according to the anti-PD1/PD-L1 trt
- Clinical value of positive immune cells for PD-L1 is debated in NSCLC
- N-linked glycosylation of PD-L1 hinders its recognition by PD-L1 antibodies

The beginning of the end?


Cancer Cell 36, 168–178, August 12, 2019 © 2019 Elsevier Inc.
In the real world, how viable an option is first line immunotherapy in NSCLC according to the PD-L1 IHC status?

- Patients with tumors > 50% PD-L1: 30% patients
- Patients with poor PS not eligible for treatment: 34% patients
- Patients with either EGFR mut or ALK/ROS1 fusion: 18% patients
- Patients with preexisting autoimmune disease: 13% patients

Patients currently eligible for immunotherapy first line in clinical routine practice:

Around 10% of stage IIIB/IV NSCLC patients

David Johnson, Chicago Oral Communication ASCO 2019

Abstract

Oncology Platform Lungscape non-small cell lung carcinoma cohort to explore this issue. PD-L1 expression was assessed via immunohistochemistry on tissue microarrays (up to four cores per case), using the DAKO 28-8 immunohistochemistry assay, following a two-round external quality assessment procedure. At the two external quality assessment rounds, tissue microarray scoring agreement rates between pathologists were: 73% and 81%. There were 2008 cases with valid immunohistochemistry tissue microarray results (50% all cores evaluable). Concordant cases at 1, 25, and 50% were: 85, 91, and 93%. Tissue microarray core results were identical for 70% of cases. Sensitivity of the tissue microarray method for 1, 25, and 50% was: 80, 78, and 79% (specificity: 90, 95, 98%). Complete agreement between tissue microarrays and whole sections was achieved for 60% of the cases. Highest sensitivity rates for 1% and 50% cutoffs were detected for higher number of cores. Underestimation of PD-L1 expression on small samples is more common than overestimation. We demonstrated that classification of PD-L1 on small biopsy samples does not represent the overall expression of PD-L1 in all non-small cell cancer carcinoma cases, although the majority of cases are 'correctly' classified. In future studies, sampling more and larger biopsies, recording the biopsy size and tumor load may permit further refinement, increasing predictive accuracy.
Comparative study of the PD-L1 status between surgically resected specimens and matched biopsies of NSCLC patients reveal major discordances: a potential issue for anti-PD-L1 therapeutic strategies

M. Ilie¹,², E. Long-Mira¹,², C. Bence¹, C. Butori¹, S. Lassalle¹,², L. Bouhlel²,³, L. Fazzalari², K. Zahaf¹, S. Lalvée¹, K. Washetine¹, J. Mouroux²,⁶, N. Vénissac⁵, M. Poudenx³, J. Otto⁶, J. C. Sabourin⁷, C. H. Marquette²,³, V. Hofman¹,²,⁴ & P. Hofman¹,²,⁴

PD-L1 clone, SP142
CDx
IVDs

This way

Analytical validation
Clinical validation (clinical trials)

That way

LDTs

Available platforms
Less expensive
Harmonization across disease sites
I-O & Chemotherapy in first line for non squamous cell carcinoma of the lung

Patients wild type for \textit{EGFR/ALK(\&ROS1/BRAFV600)}

I-O in first line for non squamous cell carcinoma of the lung

Patients wild type for \textit{EGFR/ALK/ROS1/BRAFV600} and with > 50\% of tumor cells positive for PD-L1
ROAD MAP

Brief background in thoracic oncology

Which samples and which limitations?

Which biomarkers?

Which technology?

Take away message
IHC versus FISH (versus RNA seq)

**ALK status:** ALK IHC +++ stop; ALK IHC + or ++ go to ALK FISH

**ROS1 status:** ROS1 + to +++ go to ROS1 FISH
Targeted *versus* (large) panel sequencing?

Specific-based PCR *versus* Next Generation Sequencing

According to:
- Urgent need
- Technology available
- Sample quantity/quality
Laboratory of Clinical and Experimental Pathology (Nice, Fr)

**Targeted Sequencing**
- Pyromark24
- COBAS 480
- Rotor-Gene Q

**Next Generation Sequencing**
- Thermo S5
- Genereader (1.1)
- Miseq

**Digital PCR**
- Idylla
**COBAS 480**

Plasma TAT < 24h

Tissue TAT < 24h

**Idylla**

Plasma TAT < 24h

Tissue TAT < 3 days

**Genereader (1.1)**

Plasma TAT ~ 3 days

Tissue TAT ~ 3 days

**Thermo S5**

Plasma TAT ~ 10 days

Tissue TAT ~ 10 days
Different strategies according to:

- The « quality » of the biopsies
- The urgent need for getting the results
- The budget and economic system
Different strategies according to:

- The quality of the biopsies
- The urgent need for getting the results
- The budget and economic system

Immuno-molecular testing algorithms

NGS reflex

IHC PD-L1 (22C3/Ventana Ultra)

IHC ALK (D5F3/Ventana)
IHC ROS1 (D4D6/Ventana)
IHC BRAFV600E (VE1/Ventana)

EGFR testing

NGS

How to optimize?
Diagnosis biomarkers
- TTF1
- P40
- Keratin

Predictive biomarkers
- ALK
- ROS1
- BRAF
- PD-L1

MIDI panel
- TTF1
- p40
- PD-L1
- Keratin

MIMP panel
- ALK
- ROS1
- BRAF

EGFR testing

5 mandatory biomarkers obtained in 3 working days following the biopsy

Lung adenocarcinoma

Lung squamous cell carcinoma
Tumor Mutation Burden: Is It Ready for the Clinic?

Yasushi Goto, National Cancer Center Hospital, Tokyo, Japan
## Tissue Tumor Mutation Burden 1L (CM227)

<table>
<thead>
<tr>
<th>Randomized groups</th>
<th>Median OS, months</th>
<th>HR</th>
<th>HR (95% CI)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>NIVO + IPI (n = 583)</td>
<td>Chemo (n = 583)</td>
<td>Stratified</td>
</tr>
<tr>
<td>All randomized (N = 1166)</td>
<td>17.1</td>
<td>13.9</td>
<td>0.73</td>
</tr>
<tr>
<td>PD-L1 &lt; 1% (n = 373)</td>
<td>17.2</td>
<td>12.2</td>
<td>0.62</td>
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<tr>
<td>PD-L1 ≥ 1% (n = 793)</td>
<td>17.1</td>
<td>14.9</td>
<td>0.79&lt;sup&gt;a&lt;/sup&gt;</td>
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</tbody>
</table>

### Additional exploratory subgroups analyses<sup>b,c</sup>

<table>
<thead>
<tr>
<th>PD-L1</th>
<th>Median OS, months</th>
<th>HR</th>
<th>Unstratified</th>
<th>Unstratified</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–49% (n = 396)</td>
<td>15.1</td>
<td>15.1</td>
<td>0.94</td>
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</tr>
<tr>
<td>≥ 50% (n = 397)</td>
<td>21.2</td>
<td>14.0</td>
<td>0.70</td>
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<table>
<thead>
<tr>
<th>TMB&lt;sup&gt;d&lt;/sup&gt; (mut/Mb)</th>
<th>Median OS, months</th>
<th>HR</th>
<th>Unstratified</th>
<th>Unstratified</th>
</tr>
</thead>
<tbody>
<tr>
<td>low, &lt; 10 (n = 380)</td>
<td>16.2</td>
<td>12.6</td>
<td>0.75</td>
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</tr>
<tr>
<td>high, ≥ 10 (n = 299)</td>
<td>23.0</td>
<td>16.4</td>
<td>0.68</td>
<td></td>
</tr>
</tbody>
</table>

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Does Tumor Mutational Burden dead...or not?

Some similar challenges than for PD-L1 IHC evaluation:

- Needs for comparing the different available commercially panels (sensitivity & specificity)
- Needs for doing intra laboratory assessment
- Needs for doing inter laboratory assessment
- Needs to get an accreditation for using TMB in routine clinical practice
- Needs to evaluate the costs
- Needs to assess the turnaround time for getting results

But:

- Different panels (size, number and type of genes and mutations)
- Different approaches (hybrid capture, amplicon based sequencing)
- Different devices
- Different turnaround times according to the sequencing system
- Different costs
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Which samples and which limitations?

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Take away message
Which perspectives?
Biomarkers

Response

Resistance

Toxicity

Hyperprogression
e.g. EGFR, ALK, ROS1

e.g. IHC PD-L1, TMB
STK11/LKB1 & KEAP1 genomic alterations and I-O resistance in non squamous NSCLC

**STK11** genomic alterations are associated with inferior clinical outcomes with PCP in non-squamous NSCLC

![STK11 genomic alterations graph]

- HR 1.58 (95% CI 1.20-2.08) 
P=0.0032, log-rank test
- HR 1.57 (95% CI 1.11-2.21) 
P=0.0113, log-rank test

**KEAP1** genomic alterations are associated with inferior clinical outcomes with PCP in non-squamous NSCLC

![KEAP1 genomic alterations graph]

- HR 2.03 (95% CI 1.23-3.32) 
P=0.005, log-rank test
- HR 2.83 (95% CI 1.73-4.68) 
P=0.0001, log-rank test

Skoulidis F et al, ASCO 2019
Evolutionary divergence of HLA class I genotype impacts efficacy of cancer immunotherapy

Diego Chowell\textsuperscript{1,2,\star}, Chirag Krishna\textsuperscript{2,\star}, Federica Pierini\textsuperscript{4,\star}, Vladimir Makarov\textsuperscript{1,2}, Naiyer A. Rizvi\textsuperscript{5}, Fengshen Kuo\textsuperscript{\circ}\textsuperscript{2}, Luc G. T. Morris\textsuperscript{2,6}, Nadeem Riaz\textsuperscript{2,7}, Tobias L. Lenz\textsuperscript{8,10}\textsuperscript{\star} and Timothy A. Chan\textsuperscript{1,2,7,8,10}\textsuperscript{\star}
TCR Convergence, Evenness and CTL-4 – New Biomarker for I/O?

TCR convergence in individuals treated with immune checkpoint inhibition for cancer

Timothy Looney, Denise Topacio-Hall, Geoffrey Lowman, Jeffrey Conroy, Carl Morrison, David Oh, Lawrence Fong, Li Zhang

doi https://doi.org/10.1101/665612

Division of Hematology and Oncology, Helen Diller Family Comprehensive Cancer Center, University of California San Francisco

<table>
<thead>
<tr>
<th>Category</th>
<th>Subdefinition</th>
<th>Responder</th>
<th>Non-responder</th>
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</thead>
<tbody>
<tr>
<td>Cancer Type</td>
<td>Prostate</td>
<td>2</td>
<td>4</td>
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<tr>
<td></td>
<td>Melanoma</td>
<td>7</td>
<td>6</td>
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<tr>
<td></td>
<td>Adenocarcinoma</td>
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<td>0</td>
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<tr>
<td></td>
<td>Not Indicated</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>11</td>
<td>11</td>
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<thead>
<tr>
<th>Repertoire Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clones Detected</td>
</tr>
<tr>
<td>TCR Convergence</td>
</tr>
<tr>
<td>Clonality</td>
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