Liquid Biopsy in Lung Cancer
Summary

1. Introduction to lung cancer
2. Applications of liquid biopsy in lung cancer
3. Case studies
4. Conclusions
Introduction to Lung Cancer
1. Introduction to Lung cancer

## Background

### Percentages of new cancer cases and cancer deaths worldwide in 2018

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lung</strong></td>
<td><strong>Lung</strong></td>
</tr>
<tr>
<td>11.5% of all new cases, 2.099 million</td>
<td>11.6% of all cancer deaths, 1.5 million</td>
</tr>
<tr>
<td><strong>Breast</strong></td>
<td><strong>Colorectal</strong></td>
</tr>
<tr>
<td>10.2% of all new cases, 1.8 million</td>
<td>7.7% of all cancer deaths, 1.3 million</td>
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<tr>
<td><strong>Stomach</strong></td>
<td><strong>Liver</strong></td>
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<tr>
<td>5.2% of all new cases, 1.1 million</td>
<td>8.2% of all cancer deaths, 712 000</td>
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</table>

- Most common cancer worldwide
- **Poor survival:** majority of lung cancer patients present with **advanced incurable disease**
  - 5yr survival (NSCLC):
    - Stage I/II 50%, III 15%, IV <3%
- Common **presentation symptoms:** Cough, fatigue, dyspnea, pain, weight loss
Evaluation of Lung Cancer

- Diagnosis requires lesion assessment via X-ray, MRI, PET/CT imaging of chest/abdomen/pelvis, brain
- Biopsy for pathologic assessment, and molecular testing
  - Biopsy of primary tumors and metastatic lesions equally suitable
  - Bone biopsy not recommended for molecular testing due to decalcification and degradation of DNA
- Metastasis common in lung, adrenal glands, liver, brain, bones
  - Symptoms are site-specific
Evaluation of Lung Cancer

- Histologic and biomarker assessment is critical for guiding therapeutic selection and assisting in predicting treatment response

  - For non-squamous histology:
    testing for EGFR mutations, ALK and ROS1 rearrangements, BRAF V600E mutation, and PD-L1 expression level

  - For squamous histology:
    testing for PD-L1 expression level
1. Introduction to Lung cancer

Evaluation of NSCLC: clinical guidelines

Histologic and biomarker assessment is critical for guiding therapeutic selection at diagnosis:

- **Adenocarcinoma**
- **Large cell**
- **NSCLC not otherwise specified (NOS)**

**CLINICAL PRESENTATION**

- Advanced or metastatic Disease

**HISTOLOGIC SUBTYPE**

- Establish histologic subtype\(^a\) with adequate tissue for molecular testing (consider rebiopsy\(^b\) if appropriate)
- Smoking cessation counseling
- Integrate palliative care\(^a\) (See NCCN Guidelines for Palliative Care)

**TESTING**

- Molecular testing
  - *EGFR* mutation testing (category 1)
  - *ALK* testing (category 1)
  - *ROS1* testing
  - *BRAF* testing
  - Testing should be conducted as part of broad molecular profiling\(^i\)
  - *PD-L1* testing (category 1)

**TESTING RESULTS**

- Sensitizing *EGFR* mutation positive (see NSCLC-18)
- ALK positive (see NSCLC-21)
- *ROS1* positive (see NSCLC-24)
- *BRAF* V600E positive (see NSCLC-25)
- *PD-L1* ≥50% and *EGFR, ALK* negative or unknown (see NSCLC-26)
- *EGFR, ALK, ROS1, BRAF* negative or unknown, *PD-L1* <50% or unknown (see NSCLC-27)
- Sensitizing *EGFR* mutation positive (see NSCLC-18)
- ALK positive (see NSCLC-21)
- *ROS1* positive (see NSCLC-24)
- *BRAF* V600E positive (see NSCLC-25)
- *PD-L1* ≥50% and *EGFR, ALK* negative or unknown (see NSCLC-26)
- *EGFR, ALK, ROS1, BRAF*, negative or unknown, *PD-L1* <50% or unknown (see NSCLC-28)
## Targeted therapy for advanced or metastatic disease

**Monitoring During Initial Therapy**
- Response assessment after 2 cycles, then every 2–4 cycles with CT of known sites of disease with or without contrast or when clinically indicated.

**Maintenance Therapy**
- Continuation maintenance refers to the use of at least one of the agents given in first line, beyond 4–6 cycles, in the absence of disease progression. Switch maintenance refers to the initiation of a different agent, not included as part of the first-line regimen, in the absence of disease progression, after 4–6 cycles of initial therapy.

### Sensitizing EGFR Mutation Positive
- **First-line therapy**
  - Afatinib\(^1\)
  - Erlotinib\(^2\)
  - Dacomitinib\(^3\)
  - Gefitinib\(^4,5\)
  - Osimertinib\(^6\)
- **Subsequent therapy**
  - Osimertinib\(^7\)

### BRAF V600E Mutation Positive
- **First-line therapy**
  - Dabrafenib/trametinib\(^20\)
- **Subsequent therapy**
  - Dabrafenib/trametinib\(^21,22\)
  - PD-L1 \(\geq 50\%\)
- **First-line therapy**
  - Pembrolizumab\(^23,24\)
  - Carboplatin or cisplatin/ paclitaxel/ bevacizumab/ atezolizumab (non-squamous)\(^25\)
  - Carboplatin/ paclitaxel/ bevacizumab/ atezolizumab (non-squamous)\(^26\)
  - Carboplatin or cisplatin/ paclitaxel/ or albumin-bound paclitaxel/ pembrolizumab (squamous)\(^27\)

### ALK Rearangement Positive
- **First-line therapy**
  - Alectinib\(^8,9\)
  - Brigatinib\(^10\)
  - Ceritinib\(^11\)
  - Crizotinib\(^12,13\)
- **Subsequent therapy**
  - Alectinib\(^14,15\)
  - Brigatinib\(^16\)
  - Ceritinib\(^17\)

### ROS1 Rearangement Positive
- **First-line therapy**
  - Ceritinib\(^18\)
  - Crizotinib\(^19\)
# Emerging biomarkers for therapy selection

<table>
<thead>
<tr>
<th>Genetic Alteration (ie, Driver event)</th>
<th>Available Targeted Agents with Activity Against Driver Event in Lung Cancer</th>
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</thead>
<tbody>
<tr>
<td>High-level MET amplification or MET exon 14 skipping mutation</td>
<td>Crizotinib&lt;sup&gt;1-5&lt;/sup&gt;</td>
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</tbody>
</table>
| RET rearrangements                    | Cabozantinib<sup>6,7</sup>  
                                         | Vandetanib<sup>8</sup>         |
| ERBB2 (HER2) mutations                | Ado-trastuzumab emtansine<sup>9</sup>                                 |
| Tumor mutational burden (TMB)*        | Nivolumab + ipilimumab<sup>10</sup>  
                                         | Nivolumab<sup>11</sup>       |

*TMB is an evolving biomarker that may be helpful in selecting patients for immunotherapy. There is no consensus on how to measure TMB.*

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NCCN Guidelines versión 1 2019
Applications of liquid biopsy in lung cancer
Feasibility of solid biopsy in NSCLC

Suitable tissue for molecular testing continues to be a challenge:

- 15% of patients had inadequate tumor sample for molecular analysis
- Number of patients were poor candidates for biopsy due to comorbidities

Liquid biopsy could be a solution at diagnosis
2. Applications of liquid biopsy in lung cancer

“Re-biopsy” Strategy for Biomarker Testing in Advanced Stage NSCLC: Looking for changes in Actionable Oncogenes

ESMO Clinical Practice Guidelines, 2018
“Re-biopsy” Strategy for Biomarker Testing in Advanced Stage NSCLC: Looking for changes in Actionable Oncogenes

Adapted from: Gandara. ASTRO/ASCO/IASLC Symposium on Molecular Testing, 2012.
2. Applications of liquid biopsy in lung cancer

**Feasibility of rebiopsy in NSCLC treated with EGFR TKI**

*Rebiopsy for patients with non-small-cell lung cancer after epidermal growth factor receptor-tyrosine kinase inhibitor failure*

Ineligible for rebiopsy 45/120 (38%):
- Inaccessible tumor sites: n=19 (18 brain metastases and 1 lung lesion <20mm),
- Physician decision: n=10;
- Patient Refusal n=6;
- Unknown n=10

*How can we overcome this obstacle?*

Liquid biopsy

Liquid biopsy is a test to detect **circulating biomarkers for cancer** in different body fluids (blood, saliva and urine...)

- Circulating Tumor cells (CTCs)
- Circulating Tumor DNA (ctDNA)
- Exosomes
What is Circulating Tumor DNA?

- **Features**
  - Originates from tumor cells
  - Derives from necrotic and apoptotic cells and secretion
  - Is fragmented DNA (< 200 bp)

- **Snapshot” of the entire tumor**
  - Has a short half-life (< 2 hours)
  - Harbors the somatic genomic alterations of and metastatic lesions found in a patient’s.

- **Only for metastatic cancer patients**
  - ctDNA concentration depends on location, size and vascularity of the tumor

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Science Translational Medicine  19 Feb 2014
ctDNA is not the only entity in blood with information about tumor genetic alterations

**Exosomes**: tumor cells secrete these extracellular vesicles, which contain constituents of the cell of origin, including RNA

- The analysis of this RNA provides us with information regarding **gene fusions, aberrant splicing forms** and mutations due to RNA editing

Redzic et al., 2014
### Why liquid biopsy?

<table>
<thead>
<tr>
<th>Safety and convenience</th>
<th>Tissue biopsy</th>
<th>Liquid biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Invasive and uncomfortable</td>
<td>Minimally invasive, few risks</td>
</tr>
<tr>
<td></td>
<td>Costly</td>
<td>Cheaper</td>
</tr>
<tr>
<td></td>
<td>Time intensive</td>
<td>Faster</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Accurate information</th>
<th>Tissue biopsy</th>
<th>Liquid biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical modification in DNA</td>
<td>No chemical artefact</td>
<td></td>
</tr>
<tr>
<td>Not representative of current tumor mutational status</td>
<td>Improve the assessment of tumor heterogeneity</td>
<td></td>
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<tr>
<th>Monitoring possible</th>
<th>Tissue biopsy</th>
<th>Liquid biopsy</th>
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<tbody>
<tr>
<td>Follow-up of a patient not feasible</td>
<td>Allow multiple measurements</td>
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**OncoDNA**

What is liquid biopsy? / Introduction to liquid biopsy in a Specialized Cancer Center
2. Applications of liquid biopsy in lung cancer

Which solutions and when to use them?

**IF NO SOLID BIOPSY:**

**OncoSELECT™**

CANCER-SPECIFIC SOLUTION FROM A LIQUID BIOPSY SAMPLE

OncoSELECT is a fast, minimally invasive analysis of circulating tumor DNA from a blood sample.

It is the perfect tool to identify therapeutic solutions for cancer patients not able to get their tumor biopsied, to monitor the progression of their cancer and to detect treatment resistance as soon as it appears. (before first line treatment to check the heterogeneity of the disease or acquired mutation leading to resistance).

**MATERIAL**

- 2 blood samples (2 Streck tubes of 10 ml each)

**RECOMMENDED FOR:**

Metastatic cancers of the following type:
- Non Small Cell Lung Cancer
- Breast Cancer (HER1+ or HER2+)
- Colorectal Cancer

**RESPONSE LIQUID MONITORING:**

**OncoTRACE™**

TRULY PERSONALIZED MONITORING SOLUTION FROM LIQUID BIOPSY SAMPLE

OncoTRACE is a test based on circulating tumor DNA (ctDNA) in liquid biopsies (principally blood). It is used to monitor the progression of the tumor (burden of the disease) and to detect lack of response or resistance to treatment as soon as it appears. This assay is customized for each patient, as it contains personal cancer-specific markers and variants identified in a previous genomic analysis.

**ctDNA can be used as a non-invasive tool to monitor the disease evolution during treatment.**

**OncoTRACE can:**

- Predict a relapse sooner than conventional imaging technologies
- Detect resistance mutations to anticipate therapy changes
- Predict sensitivity to new therapies

**MATERIAL**

- 2 blood samples (2 Streck tubes of 10 ml each)

**RECOMMENDED FOR:**

Any stage IV metastatic tumour type with an established genomic profile
OncoSELECT

- Designed to interrogate cancer-specific key biomarkers
- For advanced NSCLC patients (stage IV)
- Identify mutations associated with sensitivity and resistance to targeted therapies
- Information on tumor dynamics in real-time

- Optimized process to achieve greater sensitivity and specificity
  - > 99% sensitivity
  - > 99% specificity
  - Coverage of 15000x
- Detect all classes of alterations in DNA (CNV, insertion/deletion, SNV), RNA (fusions) and exon skipping.

- Requires only two 8mL tubes of blood
- Turnaround time 7-10 days
2. Applications of liquid biopsy in lung cancer

**OncoSELECT NSCLC Cancer-Driving mutations detected**

- KRAS 20%
- EGFR 15%
- MEK inhibitors (MEK162 in clinical trial)
- NRAS 1%
- HRAS 1%
- PI3KCA 1%
- MAP2K1 1%
- RET fusion 2%
- ROS fusion 2%
- MET amp 1%
- MetEx14 4%
- ERBB2 3%
- BRAF 7%
- EGFR TKIs (erlotinib, gefitinib, afatinib, osimertinib)
- ALK and/or ROS fusion (crizotinib, cabozantinib, brigatinib, alectinib, ceritinib, lorlatinib)
- cMET inhibitors (crizotinib, cabozantinib)
- HER2 inhibitors (pertuzumab, traztuzumab, lapatinib)
- BRAF inhibitors (dabrafenib, trametinib, vemurafenib)
- RET inhibitors (cabozantinib)

➔ Driver mutations were identified in more than half of all cases of adenocarcinoma.

Genetic mutations associated with treatment in lung adenocarcinoma
Non-invasive liquid biopsy-based test that allows a personalized and sensitive monitoring of the patient’s response to the treatment from circulating tumor free DNA to identify:

- Tumor’s recurrence
- Potential mechanisms of resistance
- New possibilities of treatment

**PERSONALIZED LIQUID BIOPSY**

Next-generation sequencing

- Each test is designed on the unique molecular signature of your tumor, coming from a previous genomic sequencing done through a solid or liquid biopsy analysis
- Targets more individual-specific mutations (12) leading to a higher probability of ctDNA detection to monitor recurrence
- In addition 40 genes associated with mutations (SNV, Indel and CNV) of resistance and sensitivity to targeted therapies
Case studies

Liquid biopsy in Lung Cancer
3. Case studies

Liquid biopsies in NSCLC have different uses

a) Assessment of specific mutations that can direct treatment management

b) Monitoring of the response and resistance to therapy
a) Assessment of specific mutations that can direct treatment management

The patient was previously treated by erlotinib and osimertinib based on the mutations in EGFR gene and with pembrolizumab. After a new relapse, an OncoSELECT analysis was performed, so the oncologist was able to know that a BRAF mutation had appeared predicting sensibility to others targeted therapies: dabrafenib/trametinib.
In October 2014, no EGFR mutations were found by local laboratory. The patient started treatment with cisplatin 150 mg + gemcitabine 2000 mg in combination.

Patient: 61 yo, male, non smoker
Diagnosis: NSCLC
Diagnosis date: October 2014
Previous systemic therapies: Gemcitabine + cisplatine and docetaxel
One year later, the CT showed the presence of the same metastases. The patient started treatment with 3 cycles of docetaxel 75 mg / m².
In April 2015, a progression was detected and the OncoDEEP study was requested.

2 activating mutations were detected in EGFR gene.
In July 2015, treatment with afatinib began, showing good tolerance to treatment.
b) Monitoring treatment response

Patient: 61 yo, male, non smoker
Diagnosis: NSCLC
Diagnosis date: October 2014
Previous systemic therapies: Gemcitabine + cisplatine and docetaxel

The 4th OncoTRACE monitoring studies performed after the start of treatment show response to treatment. The mutations in blood disappeared.
b) Monitoring treatment response

Patient: 61 yo, male, non smoker
Diagnosis: NSCLC
Diagnosis date: October 2014
Previous systemic therapies: Gemcitabine + cisplatine and docetaxel

After 16 months of treatment with afatinib, the 6th OncoTRACE revealed the presence of the acquired EGFR T790M resistance mutation (1%). The CT didn’t show disease progression so the current treatment was continued.

However, a closer monitoring was programmed.
In the 8th OncoTRACE 3 mutations were detected in blood (EGFR G719S, 1%, EGFR S768I 0.15%, EGFR T790M 0.2%). The progression was confirmed by CT.
3. Case studies

b) Monitoring treatment response

Patient: 61 yo, male, non smoker
Diagnosis: NSCLC
Diagnosis date: October 2014
Previous systemic therapies: Gemcitabine + cisplatin and docetaxel

TAGRISSO ™ (osimertinib) was the new treatment recommended.
Conclusions
6. Conclusions

➢ **High Genetic Heterogeneity** in Lung Cancer during the treatments

➢ **Biomarker assessment** is essential for guiding therapeutic selection at **diagnosis and after progression** in Advanced Stage NSCLC

➢ Liquid biopsy is the **non-invasive alternative** in patients where "re-biopsy" is not feasible for assessing specific mutations:

   ➢ Liquid biopsies can detect mutations that can direct treatment management at **any time of the disease (diagnosis and relapses)**

   ➢ Liquid biopsies can give information about **treatment resistance** and **monitoring the response** to the treatments

➢ Patients treated based on ctDNA analysis have shown **clinical outcome benefits** (Zill et al., 2016)