Best Practices in Pathology: Are We There Yet?

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Department of Translational Molecular Pathology
Anderson Clinical Faculty Chair for Cancer Treatment and Research
M. D. Anderson Cancer Center
Paradigms in Lung Cancer Molecular Pathology - 2015

• Histology subtyping of lung cancer and NSCLC is clinically important

• Molecular abnormalities can be used to direct targeted therapy and improve patients’ outcomes

• Mechanisms of resistance have been identified and new therapies have been developed

• Liquid biopsy is an option for molecular testing

• Immunotherapy is a new therapeutic option
Paradigms in Lung Cancer Molecular Pathology - 2015

- Histology subtyping of lung cancer and NSCLC is clinically important
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- Mechanisms of resistance have been identified and new therapies have been developed
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- Immunotherapy is a new therapeutic option
The goal is to provide precise histology diagnosis in all cases.
Types of Tumor Specimens In Lung Cancer

Surgical Resection

Histology

Advanced Tumor

Core Needle Biopsy (CNB)

Fine Needle Aspiration (FNA)

Endobronchial Ultrasound (EBUS) or Pleural Fluid

Formalin-fixed and Paraffin-embedded (FFPE)

Alcohol-fixed

Alcohol-fixed – Cell Block
Diagnostic Algorithm for Small Biopsy and Cytology Specimens

Tumor Positive

Biopsy

Cytology

SCLC

LCNEC

Squamous

Adenoca

NSCLC-NOS

Morphology

Morphology IHC NE (+)

Morphology IHC p63/p40 (+)

Morphology IHC TTF1 (+)

Morphology IHC (-)
NSCLC of the Lung
Differential Diagnosis

No clear ADCA or SCC morphology:
NSCLC - NOS

ADCA
TTF-1 +
Mucin +
Napsin +

Limited
Immunohistochemistry

SCC
p40 +
p63 +
CK 5/6 +

Molecular Testing
Paradigms in Lung Cancer Molecular Pathology - 2015

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Non-Small Cell Lung Carcinomas (NSCLC) Show High Number of Somatic Mutations

Lawrence et al., Nature, 2013

TCGA, Squamous Cell Carcinoma


TCGA, Adenocarcinoma

EA Collisson et al., (TCGA), Nature, 2014
Molecular Testing for NSCLC - 2012

Adapted from W. Pao and N Girard, Lancet Oncol, 2011
### Lung Cancer Targeted Therapy Landscape Change – 2015

#### Adenocarcinoma

<table>
<thead>
<tr>
<th>% Prevalence</th>
<th>Available TKIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td>Erlotinib/Gefitinib/Afatinib</td>
</tr>
<tr>
<td>4%</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>2%</td>
<td>Crizotinib</td>
</tr>
<tr>
<td>5%</td>
<td>Met inhibitors</td>
</tr>
<tr>
<td>2%</td>
<td>Ret inhibitors</td>
</tr>
<tr>
<td>5%</td>
<td>PI3K inhibitors</td>
</tr>
<tr>
<td>1%</td>
<td>Her2 inhibitors</td>
</tr>
</tbody>
</table>

- *EGFR mutation*
- *ALK-EML4 fusion*
- *ROS1-FIG fusion*
- *MET amplification*
- *KIF5B-RET fusion*
- *PI3KCA mutation*
- *HER2 mutation*

#### Squamous Cell Carcinoma

- *FGFR1 amplification* 22% FGFR TKIs
- *EGFRvIII mutation* 5% EGFR TKIs
- *PI3KCA mutation* 4% PI3KCA inhibitors
- *DDR2 mutation* 3% Dasatinib & Nilotinib

#### NSCLC

- *PD-L1/PD-1 Activation* ~30% PD-L1/PD-1 Imhibitors
EGFR Mutations in Lung Cancer (~15%)

- Deletions – 46%
- Duplications/Insertions – 9%
- L858R – 39%

Sanger Sequencing (sensitivity: ~20% mutant allele)

- Biopsy
  - FFPE
  - Frozen
- Cytology
  - Smears
  - Cell blocks (FFPE)
**EGFR Mutations in Lung Cancer**

**IPASS Trial**

**EGFR mut ( + )**

- Gefitinib (n=132)
- Carboplatin/Paclitaxel (n=129)

HR = 0.48
P<0.0001

**EGFR Mut ( - )**

- Gefitinib (n=91)
- Carboplatin/Paclitaxel (n=85)

HR = 2.85
P<0.0001

Treatment by subgroup interaction test, p<0.0001

EGFR Mutations in Lung Cancer

Reguart et al, Future Oncol, 2015

EGFR Tyrosine Kinase Structure

EGFR Sensitizing Mutations

<table>
<thead>
<tr>
<th>Exon 18 (nucleotide-binding loop)</th>
<th>Exon 19</th>
<th>Exon 20</th>
<th>Exon 21 (activation loop)</th>
</tr>
</thead>
<tbody>
<tr>
<td>888, 728</td>
<td>729</td>
<td>761, 762</td>
<td>823, 824, 875</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exon</th>
<th>2</th>
<th>5</th>
<th>7</th>
<th>13</th>
<th>16</th>
<th>17</th>
<th>18–21</th>
<th>22–24</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGF binding</td>
<td>EGF binding TM</td>
<td>Tyrosine kinase</td>
<td>Autophosphorylation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
EGFR Mutations in Lung Cancer and Overall Survival – EGFR TKI

Log-rank p=0.01

- EGFR exon 19 deletion (n=23)
- EGFR L858R (n=11)

1-yr OS | median
---------|--------
Exon 19 del | 90% | 34 mo
L858R | 44% | 8 mo

EGFR Mutations in Lung Cancer and Outcome – EGFR TKI

Time to Progression

Overall Survival
EML4-ALK Fusion in NSCLC (~6%)

FISH Test: “Break-apart Probe”

Positive Case: >15% Cells Positive (50-100 cells)

Positive Cell: Two signals separation

ALK Immunohistochemistry (Clone D5F3)

- **Biopsy**
  - FFPE

- **Cytology**
  - Cell blocks (FFPE)

EML4-ALK Fusion (+)  EML4-ALK Fusion (−)

Courtesy of Dr. Y. Yatabe
Diagnostic Algorithm for Small Biopsy and Cytology Specimens

Tumor Positive

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Cytology

SCLC

LCNEC

Squamous

Adenoca

NSCLC-NOS

Molecular Testing:

*EGFR* mutation, 
*ALK* and *ROS1* Fusion
Practical Considerations for Molecular Testing of Lung Cancer

- **Test**: EGFR (exons 18-21) mutations and ALK and ROS1 fusion, not specific assay (Targeted NGS Panels; cfDNA in selected cases)

- **Histology**: All tumors w/adenocarcinoma component, and in small samples NSCLC-NOS and other histologies (incomplete sampling).

- **Specimen**: Upfront collection of as much tissue as possible at diagnosis.

- **Consider re-biopsy**: (consider cf DNA)
  - If diagnostic sample is inadequate for molecular testing
  - At time of recurrence, or disease progression on targeted therapy

- **Metastasis vs. primary**:
  - Most accessible site (tissue quality is more important)
  - Test metastasis if developed after therapy
Practical Considerations for Molecular Testing of Lung Cancer (Cont’)

- **Parallel Testing (panels), no Sequential Testing**
- **Samples availability for testing:**
  - In house: less than 24 hours
  - Outside: less than 3 days
- **Quality control by pathologist:**
  - At least 500 cells
  - 50% tumor (vs. no-malignant) cells, and gross dissection recommended for enrichment – 10% using NGS (10 ng)
  - 50 cells/slide
- **Report:**
  - 10 days max
  - Indicate platform
  - Indicate suboptimal fixation in the report

Evolution of Types of Mutation Assays

Uniplex

- PCR-based Sanger Sequencing
- PCR-based Pyrosequencing®
- Real-time PCR DxS® Test

Multiplex

- PCR-based SNaPshot® (Applied Biosystem)
- PCR-based Mass ARRAY SNP Sequenom, Inc
- Next-Generation of Sequencing (NGS) Panels: mutations, amplification and rearrangements
Lung Cancer Mutation Consortium-1 (LCMC-1)

1,000 Adenocarcinomas
13 US Institutions

Mutations (8 genes/115 Assays)
Sequenom™ and Snap Shot™
- AKT1
- BRAF
- EGFR
- HER2
- KRAS
- MEK1
- NRAS
- PIK3CA

FISH (2 genes)
- MET amplification
- EML4-LK fusion

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Median Survival Yrs. (95% CI)</th>
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<tbody>
<tr>
<td>Driver, no targeted therapy (A)</td>
<td>318</td>
<td>2.4 (1.9-2.9)</td>
</tr>
<tr>
<td>Driver, targeted therapy (B)</td>
<td>260</td>
<td>3.6 (3.1-4.5)</td>
</tr>
<tr>
<td>No driver (C)</td>
<td>360</td>
<td>2.2 (1.8-2.5)</td>
</tr>
</tbody>
</table>

M Kris et al, JAMA, 2014
Next Generation Sequencing Platforms
MDACC Clinical Laboratory

- **Ion Torrent (Life Technologies)**
  - Semiconductor based detection of pH change

- **Illumina**
  - Flow cell based, 4-color optical imaging of fluorescent labeled nucleotides

<table>
<thead>
<tr>
<th>PGM</th>
<th>Proton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Gene Panels (1.5 – 2.0 Gbases/run)</td>
<td>Large gene panels and Whole Exome Sequencing (10 Gbases/run)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MiSeq</th>
<th>HiSeq</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Gene Panels (1.5 – 2.0 Gbases/run)</td>
<td>For Whole Exome and Genome Sequencing (600 Gbases/run)</td>
</tr>
<tr>
<td>Human Genome: 3 Gb</td>
<td>Whole Exome: 30 Mb</td>
</tr>
</tbody>
</table>

*Courtesy of Dr. Kenneth Aldape, MD Anderson Cancer Center, Houston, USA*
NGS for Mutation Analysis Samples Using FFPE Tumor Tissues at MDACC

MD Anderson Cancer Center
3,354 cases (Oct/13)
46-50 genes
(new 409 gene panel)

Non-small Cell Lung Cancer

50 genes; N=262 cases (chemonaive)

G. Simon et al, WCLC 2013

267 genes; N=176 cases (refractory)

J. Izzo et al, 2015

Lung 18.1%
Breast 18.9%
Melanoma 22.8%

Courtesy of Dr. K. Aldape, 2013
Next Generation Sequencing Platforms for Diagnosis
Applications and Adequacy for Genomic Testing

• Targeted NGS identifies mutation, amplification and translocations in panel of genes and exons (10 to ~300)
• Applicable to FFPE tissues (as low as 10 ng) with as low as 10% malignant cells
• Adequacy assessment of tissues is critical:
  ‣ A difficult issue; the same cases may not be so good for molecular testing
  ‣ Not unusual to make a diagnosis of lung cancer from only a few cells (e.g., FNAs)
  ‣ Bottom line:
    ▪ Processes to select and qualify clinical tumor samples must be in place
    ▪ Communication with ordering clinicians is required to work through limitations of clinical samples

G. Frampton al, Nature Biotechnology, 2013

FFPE Tumor Samples
Diagnostic Algorithm for Small Biopsy and Cytology Specimens - 2015

Tumor Positive

Biopsy

Cytology

SCLC

Morphology

LCNEC

Morphology

Squamous

Morphology

IHC p63/p40 (+)

Adenoca

Morphology

IHC TTF1 (+)

NSCLC-NOS

Morphology

IHC (-)

Tissue Qualification for Molecular Testing

Molecular Testing: NGS Targeted Panel
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Paradigm Shift in Tissue Collection for Molecular Profiling of Advanced Lung Cancer

- Enough tissue for molecular testing must be mandatory (re-biopsy).
- The sample must represent the status of the disease at the time of the treatment decision:
  - Tumor progression and metastasis
  - Modification by previous therapy
  - Mechanisms of resistance to targeted therapy

Re-biopsy/Molecular Testing

- Refractory to Chemotherapy
- Resistance to Targeted Therapy
Mechanisms of Resistance to EGFR TKIs in Lung Adenocarcinoma

Fig. 1 The frequency of observed drug resistance mechanisms.

- **EGFR**
- **KRAS**
- **MAPK**
- **PI3K**
- **Unknown**

- **BRAF**
- **ALK**
- **RET**
- **ROS1**
- **VEGF**
- **HER2**
- **EPHAB/B**
- **PDGFR**
- **FGFR**
- **INSR**
- **PI3K**
- **MAPK**
- **KRAS**
- **ALK**
- **RET**
- **ROS1**

**EGFR T790M Mutation** (49%)
- **SCLC Features** (14%)
- **PI3KCA Mut** (2%)
- **MET Ampl** (2%)
- **EMT Change** (14%)

**Modified from Sequist L V et al. Sci Transl Med 2011;3:75ra26-75ra26**
Acquired EGFR TKI Resistance in NSCLC

- Secondary mutations in \textit{EGFR}
  - T790m (exon 20)
  - Others: D761Y, L747S, T854A
- \textit{MET} amplification
- \textit{PI3KCA} mutation
- Other mechanisms:
  - Small cell lung cancer (SCLC) transformation
  - Epithelial-mesenchymal transition (EMT)
Mechanism of Resistance to EGFR TKIs: Secondary Mutation – T790M

- **EGFR T790M** mutation restores the affinity of EGFR-mutant protein for ATP to wild-type levels reducing the effect of TKIs

- Cells with T790M have a disadvantage for growth

- Tumors with T790M also have a more indolent phenotype

- Testing for this mutation requires re-biopsy and sensitive method for detection in tumor tissues (or liquid biopsy)
# Mechanisms of Resistance to EGFR TKI

<table>
<thead>
<tr>
<th>Histology</th>
<th>Adeno</th>
<th>SCLC</th>
<th>Adeno</th>
<th>SCLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>L858R</td>
<td>L858R</td>
<td>L858R</td>
<td>L858R</td>
</tr>
<tr>
<td>Genotype</td>
<td>PIK3CA</td>
<td>PIK3CA</td>
<td>PIK3CA</td>
<td>PIK3CA</td>
</tr>
<tr>
<td>EGFR TKI status</td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tumor burden</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
<td>![Graph]</td>
</tr>
<tr>
<td>Treatment</td>
<td>Erlotinib</td>
<td>Erlotinib</td>
<td>C+RT</td>
<td>C+RT</td>
</tr>
<tr>
<td>Timeline</td>
<td>2008</td>
<td>2009</td>
<td>2010</td>
<td>2010</td>
</tr>
</tbody>
</table>

**Adenocarcinoma**
- H&E
- Synaptophysin

**SCLC**
- H&E
- Synaptophysin

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Sequist L V et al. Sci Transl Med 2011;3:75ra26-75ra26
Mechanisms of Resistance to ALK TKIs in Lung Adenocarcinoma

- **ALK**
- **RET**
- **ROS1**
- **EGFR**
- **PI3K**
- **MAPK**
- **KRAS**
- **BRAF**
- **AKT**
- **VEGFR**
- **HER2**
- **EPHA/B**
- **PDGFR**
- **FGFR**
- **Unknown**

**ALK Resistance Mutations (~36%)**

**ALK Copy Number Gain (CNG) (~18%)**

**Alternate Oncogene (EGFR, KRAS) (36%)**

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Liquid Biopsy in Lung Cancer

• Non-invasive approach that detects diagnostic, prognostic and predictive biomarkers in cancer patients

• Currently, it is being tested in cancer patients with metastatic disease to deliver targeted therapy

• Applications:
  
  • Can be easily repeated to control treatment efficiency and/or the detection of genomic changes resulting from resistance to therapy (e.g., *EGFR T790M, ALK* mutations)
  
  • It is an alternative to patients with solid biopsies are inaccessible or after more than one attempt the yield was unsatisfactory
  
  • It may be able to address the issue of tumor heterogeneity
Source of DNA in the Bloodstream of Patients with Cancer

- Circulating tumor DNA (ctDNA)
- Circulating Tumor Cell (CTC)

Exosomes

- Endocytic origin – intracellular
- 40-200 nm
- Cargo loading seems to be (at least, partially) regulated event


Raposo et al, doi: 10.1083/jcb.201211138

TEM with immunogold

Atomic Force Microscopy
Battle of Clones – Role of Liquid Biopsy
Intra-tumor and Inter-tumor Heterogeneity

R. Burrell and C. Swanton, Molecular Oncology, Volume 8, 2014, 1095 - 1111

Mutational monitoring though “Liquid Biopsy”

- Circulating Tumor (ct) DNA
- Exosomes
- Circulating Tumor Cells (CTCs)
Detection of *EGFR* Mutations in Lung Adenocarcinoma using ctDNA

- *EGFR* mutations examined using digital droplet (dd)PCR using ctDNA.
- Analysis of activating *EGFR* mutations in 34 patients with mutant tumors.
- *L858R* was detected in 8/12 and *Del-19* in 9/23 patients with mutant tumors.
- Four patients treated with EGFR TKI were examined serially.

Geoffrey R. Oxnard et al. Clin Cancer Res 2014;20:1698-
Comparison Between CTC and ctDNA

Ilie et al, Ann Transl Med, 2014
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The Cancer-Immunity Cycle

CD8+ cytotoxic T cells become activated to kill tumors cells.

Tolerance occurs when the T-cell programmed-death receptor-1 (PD-1) interacts with its ligand, PD-L1, aberrantly expressed by the malignant tumor cell.

Monoclonal antibody to bind these proteins, as either α-PD-L1 or α-PD-1 abrogates this interaction, promoting effector T-cell–mediated rejection of tumor.
PD-L1 Expression Analysis in Tumor Tissues

**Immunohistochemistry**

0

1+

2+

3+

Parra et al, 2015

**Immunofluorescence**

Herbst et al, Nature 2014

- PD-L1 expresses in malignant cells and inflammatory cells (macrophages and others)
Response and Survival Are Associated With PD-L1 (IC)

### Summary of responses to MPDL3280A in paired biopsies

<table>
<thead>
<tr>
<th>Increase in PD-L1 (TC) (no. (%))</th>
<th>Increase in PD-L1 (IC) (no. (%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum SLD decrease</td>
<td></td>
</tr>
<tr>
<td>&gt;30% reduction</td>
<td>3/6 (50)</td>
</tr>
<tr>
<td>0%–30% reduction</td>
<td>3/8 (37)</td>
</tr>
<tr>
<td>0%–20% increase</td>
<td>2/9 (22)</td>
</tr>
<tr>
<td>&gt;20% increase</td>
<td>0/3 (0)</td>
</tr>
<tr>
<td>Unevaluable SLD</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td><strong>Objective response per RECIST v1.1</strong></td>
<td></td>
</tr>
<tr>
<td>Best response of PR</td>
<td>3/5 (60)</td>
</tr>
<tr>
<td>Best response of SD</td>
<td>5/12 (42)</td>
</tr>
<tr>
<td>Best response of PD</td>
<td>1/11 (9)</td>
</tr>
<tr>
<td>4/5 (80)</td>
<td></td>
</tr>
<tr>
<td>2/12 (17)</td>
<td></td>
</tr>
<tr>
<td>4/11 (36)</td>
<td></td>
</tr>
</tbody>
</table>

### Graphical Representation

- **Progression-free rate** vs **Time to progression (weeks)**
  - IHC 0 (n = 60): Median = 8.14 weeks, CI = 5.57 to 17.57 weeks, Range = 4.14 to 73.14+ weeks
  - IHC 1 (n = 34): Median = 17.14 weeks, CI = 6.00 to 43.29 weeks, Range = 0.14+ to 50.57+ weeks
  - IHC 2 (n = 23): Median = 18.14 weeks, CI = 6.00 to 48.14 weeks, Range = 1.00 to 48.57+ weeks
  - IHC 3 (n = 33): Median = 37.28 weeks, CI = 18.20 to 59.00 weeks, Range = 1.86 to 59.00 weeks
  - Unknown (n = 25): Median = 19.71 weeks, CI = 6.29 to NE weeks, Range = 0.14+ to 48.00+ weeks

*Herbst et al, Nature 2014*
PD-L1 Assessment in Lung Cancer Tissues - Challenges

- It is expressed in malignant and inflammatory cells, mostly macrophages
- The expression is heterogeneous in tumor tissues
- There are several antibodies (5H1, SP142, E1L3N®, etc.) available against different epitopes
- Some antibodies are run in specific IHC autostainer platforms
- There are different scoring systems
- The role of other immune-markers (PD-1, TILs, etc.) needs to be addressed
## PD-L1 IHC Expression (Ab E1L3N®) in Malignant Cells in Surgically Resected NSCLCs

<table>
<thead>
<tr>
<th>PD-L1 of Expression</th>
<th>Adenocarcinoma N=146</th>
<th>Squamous Cell Ca N=108</th>
<th>All N=254</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1%</td>
<td>87 (60%)</td>
<td>52 (48%)</td>
<td>139 (55%)</td>
</tr>
<tr>
<td>≥5%</td>
<td>34 (23%)</td>
<td>34 (32%)</td>
<td>68 (27%)</td>
</tr>
<tr>
<td>≥10%</td>
<td>31 (21%)</td>
<td>29 (27%)</td>
<td>60 (24%)</td>
</tr>
<tr>
<td>≥20%</td>
<td>21 (14%)</td>
<td>25 (23%)</td>
<td>46 (18%)</td>
</tr>
<tr>
<td>≥50%</td>
<td>9 (6%)</td>
<td>12 (11%)</td>
<td>21 (8%)</td>
</tr>
</tbody>
</table>

*E. Parra et. al. 2015*
NSCLC Tumor Immune-profiling

Uniplex IHC

CD3

CD8

CD4

CD45RO

CD57

PD1

FOXP3

CD68

PD-L1

Histology (H&E)

Pattern Recognition

Image Analysis

Multiplex IF

Vectra Multispectral Microscope (Perkin Elmer)

• CD4 (green)
• CD8 (red)
• PD-L1 (yellow)
• DPAI (blue)

MultiOmyx (GE Healthcare)

• CD3 (green); PD-1 (red); DAPI (blue)

E. Parra, et. al. 2014
Diagnostic Algorithm for Small Biopsy and Cytology Specimens - 2014

Tumor Positive

Biopsy  Cytology

SCLC  LCNEC  Squamous  Adenoca  NSCLC-NOS

Morphology  Morphology  Morphology  Morphology  Morphology
           IHC NE (+)  IHC p63/p40 (+)  IHC TTF1 (+)  IHC (-)

Tissue Qualification for Molecular Testing

Molecular Testing: Multiplex Analysis
[NGS Panel and Multiplex IHC for proteins]
Biomarker Discovery Platform

Gene expression profiling
- Arrays
- Nanostring/HTG
- RNA seq
- RT-PCR

Tumor genetic profiling
- NGS, whole exome/sequencing

Proteins
- Immunohistochemistry
- Immunofluorescence
- Proteomic arrays

Blood-based markers
- Serum/plasma proteins assays
- Liquid biopsy
  - CTC
  - cfDNA/RNA
  - Exosome-derived DNA/RNA

Germline genetic profiling
- SNP arrays

Precision Medicine
Thank you