MOLECULAR TESTING

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No financial disclosures

Biomarkers for therapy selection

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Biomarker</th>
<th>Drug</th>
<th>% eligible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>EGFR mutation</td>
<td>Gefitinib</td>
<td>12%</td>
</tr>
<tr>
<td>Lung</td>
<td>KRAS mutation</td>
<td>Cetuximab</td>
<td>12%</td>
</tr>
<tr>
<td>Colon</td>
<td>c-kit (c-kit)</td>
<td>Imatinib</td>
<td>12%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>BRAF mutation</td>
<td>Vemurafenib</td>
<td>42%</td>
</tr>
<tr>
<td>GIST</td>
<td>KIT/PDGFRA</td>
<td>Imatinib</td>
<td>90%</td>
</tr>
<tr>
<td>CML</td>
<td>BCR-ABL translocation</td>
<td>Imatinib</td>
<td>95%</td>
</tr>
<tr>
<td>Gastro</td>
<td>HER2 gene amplification</td>
<td>Trastuzumab</td>
<td>20%</td>
</tr>
</tbody>
</table>

PATHOLOGY CONSIDERATIONS FOR GOOD PRACTICE

- Small biopsy and cytology samples should be managed not only for diagnosis but also to maximize the amount of tissue available for molecular studies.

- Ten years ago, the use of molecular techniques to detect EGFR in lung cancer, KRAS in colon cancer and c-kit in GISTs was just starting.

- Today, this is routine and cytology frequently is the only available material to be tested, especially in lung cancer.

- Curiously, a technique that changed cancer genomics, next-generation sequencing was not mentioned at that time. This technology is rapidly replacing the classical use of PCR and Sanger sequencing, allowing to study not only DNA but also RNA alterations.
Lung Cancer and Personalized Therapy

Genomic Analysis in Lung Cancer

- Lung cancer is a molecularly heterogeneous disease and understanding its biology is crucial for the development of effective therapy.
- There are more than 300 non-synonymous mutations per lung cancer but only a minority can promote tumorigenesis.
- Establish targets: EGFR, ALK, ROS1, BRAF, and PDL1
- ESMO recommendation for genetic test: non-squamous and squamous in minimal or non-smokers

Molecular testing in NSCLC

Major problems
- Tissue limitations
- Slow turnaround time
- Cost effectiveness
Ten years ago, the idea that all of the genes altered in cancer could be identified at base-pair resolution would have seemed like science fiction.

Today, such genome-wide analysis, through sequencing of the exome or of the whole genome, is routine.

Vogelstein et al. Science 2013

Why Now?

The Human Genome Project

The Cancer Genome Project

The Molecular Tools

Screening lung cancer clinically relevant alterations

NSCLC: 40-50% diagnosed by cytology

Precision Oncology

More biology from smaller samples
Comprehensive testing with less tumor tissue

Cytology specimens are suitable for NGS

Any kind of cytological material can be used for NGS

NGS in cytology

Conclusions

- Cytopathology has established itself as independent diagnostic modality to guide clinical management in many different settings.
- While earlier studies have demonstrated that single biomarker testing is feasible on cytology, currently this information is insufficient to guide patient care.
- More recently, multi-gene mutational assays, such as NGS have gained popularity because they provide genomic information on multiple genes.
- Cytopathologist plays a key role in ensuring success of NGS in cytology by influencing pre-analytical steps and selecting adequate material.

Updated Molecular Testing Guideline for the Selection of Long Cancer Patients for Treatment With Targeted Tyrosine Kinase Inhibitors

Good Molecular only with Good Material
Tumour cell content: Which molecular test?

ONCOMINE FOCUS ASSAY

- Cases with < 20% tumor cells are reported to have >20% cells in 38% of the cases possibly causing false negative results.
- In conclusion, estimates of tumor cell percentages on stained slides are not accurate, which could result in misinterpretation of test results.
- Reliability could possibly be improved by using a training set with feedback.

Polyploidy – a challenging aspect in tumor cell quantification for molecular analysis

- Polyploidy should have been taken into account in tumor cell quantification because it can be a cause of discrepancy with AF detected by NGS.
- Morphological correlation with molecular results is essential for a correct interpretation of molecular tests.

Consistency and reproducibility of next-generation sequencing in cytopathology: A second worldwide ring trial study on improved cytological molecular reference specimens.

Molecular Cytopathology Group

- Genomic reference standards representative of routine cytology clinical practice showed highly reproducible results across all laboratories in detection of mutations down to 5% of AF despite the difference in smears staining and sequencing practices.

Clinical Integration of Molecular Results on Cytology (Post-analytical Phase)

- Reference materials: C. Schmidt and M. Salah-Talbi

Quadros et al. 2019

Cancer Cytopathology 2019

Molecular Cytopathology Group

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NGS in oncology

Science still early.
More data should be in public domain.
Many variations not clinically relevant.
Costs still need to go down.
Ethical issues in testing individual genotype.
Still unclear how to deliver information to the practitioner.

PERSONALIZED HEALTHCARE IN THE NEXT 10 YEARS

There is still lots to figure out…
Biomarkers for Immunotherapy

Checkpoint inhibitors
- Ipilimumab (CTLA4)
- Tremelimumab (CTLA4)

Checkpoint inhibitors
- Nivolumab (PD-1)
- Pembrolizumab (PD-1)
- Atezolizumab (PD-L1)
- Durvalumab (PD-L1)

As preanalytical processing varies significantly from histology specimens, especially for conventional cytology specimens, cytology specific PD-L1 protocols need to be established and validated.
Potential biomarkers for Immunotherapy

Tumor Mutation Load (TML)

• TML is a measure of the number of mutations within a tumour genome, defined as the total number of mutations per coding area of a tumour genome.
• There is large variability of in ML within tumour types, ranging from few to thousands of mutations.
• TML can be determined by whole-exome sequencing but can be inferred from sequencing a smaller panel of genes (ex. 324).

Immunogenic vs. non-Immunogenic

Clinical Trials defining a TMB threshold for ICB benefit
Three sample types can be used for EGFR and T790M mutation testing in advanced NSCLC:

1. Plasma
   - Rapid turnaround time to facilitate treatment decisions
   - Preferred sample for testing at progression
2. Tumour biopsy samples
   - Gold standard sample type for all EGFR mutation testing in advanced NSCLC
3. Cytology samples
   - Suitable alternative if a tumour biopsy sample is not available

EGFR T790M mutation testing on disease progression
CYTOLOGY IS ALWAYS PIONNER!

CYTOPATHOLOGIST NEED TO BE IN THE FRONTLINE