Next-Generation Sequencing/Biomarkers: Advanced Technology

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A biomarker is...

- “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.”

Types of Biomarkers: Prognostic vs Predictive Biomarkers

**Prognostic Marker**
- Information about disease outcome independent of treatment
- Example: EGFR mutation in NSCLC
  - Mutation+: better prognosis
  - Mutation-: worse prognosis

**Predictive Marker**
- Information on disease outcome related to a specific treatment
  - Example: EGFR mutation in NSCLC
  - Mutation+: ≈70% probability of response to EGFR TKI therapy
  - Mutation-: <5% probability of response to EGFR TKI therapy

Some biomarkers are both prognostic & predictive. Only predictive biomarkers can be used to indicate “which patients should be treated with which drug” (a targeted therapy). Predictive biomarkers can also identify patients who may be harmed by “targeted therapy.”
Wide Clinical Uses of Biomarkers

• Predictors of response
• Prognostic factors
• Screening/patient selection for clinical trial
• Surrogate endpoints
• Risk assessment
• Diagnosis
• Pharmacogenetics
• Monitoring
Biomarkers Have Significantly Improved Treatment in Patients With NSCLC

**EGFR** common mutation+

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Events, n (%)</th>
<th>Median (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afatinib (n = 204)</td>
<td>130 (64)</td>
<td>13.60</td>
</tr>
<tr>
<td>Cisplatin/pemetrexed (n = 104)</td>
<td>61 (59)</td>
<td>6.90</td>
</tr>
</tbody>
</table>

HR, 0.47; 95% CI, 0.34 to 0.65; *P* < .001

**ALK** rearrangement+

Hazard ratio for progression or death in the crizotinib group, 0.49 (95% CI, 0.37–0.64)

*P* < .001

EGFR Mutation and ALK Rearrangement Testing in All NSCLC: NCCN, ASCO, and ESMO Lung Treatment Guidelines

NCCN Guidelines Version 5.2015
Non-Small Cell Lung Cancer

SYSTEMIC THERAPY FOR METASTATIC DISEASE

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

HISTOLOGIC SUBTYPE

- Adenocarcinoma
  - Large Cell
  - NSCLC not otherwise specified (NOS)
  - EGFR mutation testing (category 1)
  - ALK testing (category 1)
  - EGFR and ALK testing should be conducted as part of multiplex/next generation sequencing

- Squamous cell carcinoma
  - Consider EGFR mutation and ALK testing especially in never smokers or small biopsy specimens, or mixed histology
  - EGFR and ALK testing should be conducted as part of multiplex/next generation sequencing

TESTING RESULTS

- Sensitizing EGFR mutation positive
  - See First-Line Therapy (NSCL-17)

- ALK positive
  - See First-Line Therapy (NSCL-18)

- Both sensitizing EGFR mutation and ALK are negative or unknown
  - See First-Line Therapy (NSCL-19)

- Sensitizing EGFR mutation positive
  - See First-Line Therapy (NSCL-17)

- ALK positive
  - See First-Line Therapy (NSCL-18)

- Both sensitizing EGFR mutation and ALK are negative or unknown
  - See First-Line Therapy (NSCL-20)

1. NCCN Treatment Guideline for NSCLC v7. 2015.
Key Technologies Driving the Development of Biomarkers

• **1st-generation/Sanger sequencing** is one of the main approaches for identifying genetic abnormalities in known driver mutations (eg. *EGFR* and *ALK*)

• Additional methods include:
  – Immunohistochemistry (IHC)
  – Fluorescence in situ hybridisation (FISH)

1st-Generation vs
Next-Generation Sequencing
1st-Generation Sequencing: Sanger’s Method

- Based on the principle of “chain termination” with the use of dideoxynucleotides (ddNTPs)
- The reaction products are separated by gel electrophoresis
- The sequence readout is determined by detection of laser-excited fluorescently labelled fragments
- Low throughput: <1 million base of DNA per day

The Contributions of 1st-Generation Sequencing

- Sequencing of the human genome (completed in early 2001\textsuperscript{2})
  - Required >12 years
  - Multiple laboratories
  - Cost > $3 billion

- Improved treatment of NSCLC\textsuperscript{3,4}
  - \textit{EGFR} sequencing to identify and select patients appropriate for EGFR-targeted TKIs
  - Sequence-based detection of \textit{EML4-ALK} translocations to select patients for crizotinib

- Limitations\textsuperscript{5}
  - Only detect the expression of selected known hotspot mutations and oncogenes
  - Do not have the ability to discover new or additional drug targets

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Next Generation Sequencing (NGS)

- Massively parallel sequencing of the broken genomic DNA$^{1,2}$
- Does not use the Sanger method$^{1,2}$
- Different platforms with different chemistries
- Very high throughput instruments$^{1,2}$
  - $>$100 gigabases of DNA sequence/day
- Applications$^{3,4}$
  - Whole genome sequencing
  - Exome sequencing
  - Transcriptome sequencing (sequencing/quantification)$^1$
  - Epigenetic studies
  - Mutation detection
    - Somatic mutation and small insertion and deletion
    - Copy number variation
    - Chromosomal rearrangement

Why NGS?

• NGS offers **novel and rapid ways** for whole genome-wide characterisation of DNA, mRNA, transcription factor regions, miRNA, chromatin structure, and DNA methylation patterns that are not possible with 1st-generation sequencing.
## Commercially Available NGS Platforms

<table>
<thead>
<tr>
<th>Basic Technique</th>
<th>Major Uses</th>
<th>Time Needed</th>
<th>Instrument Cost ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illumina</td>
<td>Whole exome sequencing; whole genome sequencing; SNP detection</td>
<td>Intermediate, 4 d fragment; 9 d paired end</td>
<td>500-900</td>
</tr>
<tr>
<td>454 Pyrosequencing</td>
<td>Targeted exon sequencing; confirmatory sequencing; SNP detection</td>
<td>Fast, &lt;1 d</td>
<td>500-700</td>
</tr>
<tr>
<td>Helicos</td>
<td>Single molecule sequencing; whole genome sequencing</td>
<td>Slowest, 8 d</td>
<td>999</td>
</tr>
<tr>
<td>SOLiD</td>
<td>Whole exome sequencing; whole genome sequencing; SNP detection</td>
<td>Slow, 7 d fragment run; 14 d paired end</td>
<td>600-700</td>
</tr>
<tr>
<td>Ion Torrent</td>
<td>Targeted sequencing projects not demanding deep sequencing</td>
<td>Fast, &lt;1 d</td>
<td>50</td>
</tr>
</tbody>
</table>
## Comparison of Traditional (Sanger) and NGS Methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Traditional</th>
<th>NGS</th>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per Base</td>
<td>High</td>
<td>Low</td>
<td>NGS</td>
</tr>
<tr>
<td>Cost per Single clinical “test” ($)</td>
<td>Low to moderate, 300-500</td>
<td>Moderate to high, 800-2,000 (estimated)</td>
<td>Traditional</td>
</tr>
<tr>
<td>Cost per 10-gene multiplex multigene test ($)</td>
<td>High, 3,000-5,000</td>
<td>Moderate, 800-2,000</td>
<td>NGS</td>
</tr>
<tr>
<td>Equipment</td>
<td>Moderate</td>
<td>High</td>
<td>Traditional</td>
</tr>
<tr>
<td>Expertise required for sequencing and data analysis</td>
<td>Moderate</td>
<td>High</td>
<td>Traditional</td>
</tr>
<tr>
<td>Can be performed using FFPE samples</td>
<td>Yes</td>
<td>Yes</td>
<td>—</td>
</tr>
<tr>
<td>Challenged by small samples, necrotic tumor, tumor heterogeneity, and low percentage of tumor sample DNA</td>
<td>Yes</td>
<td>Yes</td>
<td>—</td>
</tr>
<tr>
<td>Generally restricted to 1 gene at a time</td>
<td>Yes</td>
<td>No</td>
<td>NGS</td>
</tr>
<tr>
<td>Can easily sequence hundreds of cancer-related genes in 1 sample</td>
<td>No</td>
<td>Yes</td>
<td>NGS</td>
</tr>
<tr>
<td>Generally restricted to hot-spot analysis</td>
<td>Yes</td>
<td>No</td>
<td>NGS</td>
</tr>
<tr>
<td>Can detect deletions</td>
<td>No</td>
<td>Yes</td>
<td>NGS</td>
</tr>
<tr>
<td>Can detect translocations</td>
<td>No</td>
<td>Yes</td>
<td>NGS</td>
</tr>
<tr>
<td>Can detect gene copy number alterations</td>
<td>No</td>
<td>Yes</td>
<td>NGS</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Low</td>
<td>High</td>
<td>NGS</td>
</tr>
<tr>
<td>Accuracy</td>
<td>High</td>
<td>High</td>
<td>—</td>
</tr>
<tr>
<td>Regulatory status</td>
<td>“Gold standard”</td>
<td>Pending</td>
<td>Traditional</td>
</tr>
<tr>
<td>Turnaround time</td>
<td></td>
<td></td>
<td>Traditional</td>
</tr>
<tr>
<td>Single gene</td>
<td>Shorter</td>
<td>Longer</td>
<td>Traditional</td>
</tr>
<tr>
<td>per Multiplex multigene test</td>
<td>Longer</td>
<td>Longer</td>
<td>—</td>
</tr>
</tbody>
</table>

FFPE, formalin-fixed, paraffin-embedded; NGS, next-generation sequencing.

Current Use of NGS in NSCLC

- Understanding molecular mechanism of disease
- Discovery of novel drug targets
- Screening patients for clinical trials
High Rates of Somatic Mutation Were Seen in Adenocarcinoma of the Lung

Somatic mutations (mean 8.9 mutations per megabase)

- 230 resected adenocarcinoma of the lung were profiled and analysed as part of the Cancer Genome Atlas initiative

Aberrations in NF1, MET, ERBB2, and RIT1 occurred in ≈13% of the cases and were enriched in samples lacking an activated oncogene, suggesting a driver role for these events in certain tumours.

Significantly Mutated Genes in SqCC of the Lung

- 178 squamous cell carcinomas of the lung were profiled and analysed as part of the Cancer Genome Atlas initiative

Alterations in targetable oncogenic pathways in SCC of the lung

- PI3/RTK/RAS signalling altered in 69% of patients
- Alterations in the PI3K/AKT pathway genes were mutually exclusive with EGFR alterations

NGS in Biomarker-driven Trials in NSCLC
Fresh tumour biopsy or archival FFPE tumour from eligible patients with stage IIIB or IV lung SCC whose disease has progressed on first-line therapy is evaluated using NGS (FoundationOne) and, in some cases, molecular assays (e.g., IHC-based), carried out in a CLIA-certified laboratory for the presence of drug-specific biomarkers relevant to lung SCC that may serve as targets for drugs currently under study in Lung-MAP. Results are returned within 10–14 days of tissue submission.

Second-line Therapy in Squamous Lung Cancer (LUNG-MAP): Schema for Sub-studies

*Archival FFPE tumour, fresh core needle biopsy (CNB) if needed.
TT = targeted therapy; CT = chemotherapy (docetaxel or gemcitabine);
TKI = tyrosine kinase inhibitor (erlotinib).

*NGS to understand the mechanisms of drug resistance and genomic evolution.

NGS in NSCLC: Key Considerations and Future Perspectives
Considerations in the Use of NGS in the Management of Cancer, Including NSCLC

• **Sampling**¹
  - High intratumour heterogeneity presents challenges¹
  - Dynamic changes during tumour progression or response to therapy
  - The need to improve regulatory system to ensure the quality of conducting genetic and genomic testing (quality and quantity of tumour tissues)

• **Analysis**¹
  - Distinguish between driver mutation from passenger genetic abnormalities
  - Large computer capacity needed to properly and efficiently analyse the huge amount of data generated

• **Close collaboration among all stakeholders**¹
  - Multidisciplinary health care providers (surgeons, pulmonologists, radiologists, pathologists, translational scientists, medical oncologists, insurers, regulatory agencies and patients are required for successful clinical implementation of NGS in genotyping and genomic testing¹

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Future Prospects for NGS in NSCLC

• With steady advances in NGS and associated technologies, costs of NGS are expected to decrease and speed and processing accuracy are expected to increase\(^1\)

• NGS technology will become increasingly accessible to researchers and clinicians and will continue to transform cancer genomics, leading to identification of all the major alterations in the cancer genomes\(^1\)

• The incorporation of NGS in patient management holds the promise of advancing personalised cancer treatment, with the goal of maximizing efficacy and minimizing toxicity\(^2\)

Summary/Conclusion

• First-generation sequencing has identified key driver mutations in subpopulations of patients with NSCLC, and their inhibitions have resulted in significant improvement in clinical outcomes in these patients

• However, there are inherent properties/characteristics that have limited their use

• NGS offers a novel and rapid ways for large-scale whole genome-wide profiling and analysis with the capacity for better biomarker identification

• Its increased use in translational research and clinical development holds the promise of improving our current understanding of oncogenesis and advancing personalized cancer treatment
City of YOKOHAMA will host WCLC 2017
Please come and join!

I thank you for your kind attention!
Importance of Patient Selection (Enrichment): Without Selection, a Positive Treatment Effect May Be Missed

(Courtesy of Gwen Fyfe)

Mathematical Modeling

100% of patients show treatment effect

50% of patients show treatment effect

25% of patients show treatment effect