NGS: A case based review

Next Generation Sequencing:
Current Technology and Applications

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Objectives

• Present actual cases to illustrate principles of molecular analysis of tumors by next generation sequencing (NGS).

• What molecular abnormalities can NGS detect?

• What substrates/ tissue/ cell types can be reliably analyzed and what cannot?

• How does NGS aid in the current management of cancer patients?
Next Generation Sequencing

Also known as massive parallel sequencing

**Strong Growth Underway**

**NGS Market Adoption**

**US Market**

- **2017 NGS Testing**
  - 272 Clinical genetic testing labs
  - 5.5 MM Clinical genetic tests conducted
  - 1.1 MM NGS tests conducted

**Molecular Diagnostics Market**

**In-House Testing**

Molecular diagnostic market size is projected to reach $10.12 Billion from $6.54 Billion in 2016, at a CAGR of 9.1%.

*As most diagnostic tests are performed in-house, the hospital & academic laboratories segment is expected to dominate the market.*

Source: ReportanReports

**Next Generation Sequencing**

Global oncology based molecular diagnostics market is expected to reach $3.39 Billion by 2022.

*Sequencing is estimated to be the fastest growing segment with CAGR estimated at over 19%.*

Source: Grand View Research

Courtesy of Dr. Eric Loo
Background of NGS

- NGS allows massive production of tens of millions of short sequencing fragments, high-throughput and low cost
- Dependent on the existence of a reference sequence and requires bioinformatics processing for alignment
- Enables testing of many genes in a single assay with very small amount sample nucleic acid
Clinical Applications of NGS

- Personalized/precision medicine
- Inherited vs. acquired diseases
- Tumor profiling
- Choice of therapy
Personalized/Precision Medicine

Administer
- the right drug
- at the right dose
- to the right person
- at the right time

- Risk of disease
- Response to treatment
- Side effects
Tumor Profiling

(Comprehensive NGS test)
Choice of Therapy

- Matching the treatment to pathophysiology of disease
- Genetic variants that predict response
- Patterns of gene expression that reveal disease subtypes

**Patient 1**
Disease: Melanoma  
Gene: BRAF  
Variant: V600E Mutation

**Patient 2**
Disease: Colorectal Cancer  
Gene: BRAF  
Variant: V600E Mutation

**Patient 3**
Disease: Colorectal Cancer  
Gene: BRAF  
Variant: D594G Mutation

**Potential Therapeutic Strategy:**
- Patient 1: vemurafenib + cobimetinib or dabrafenib + trametinib
- Patient 2: vemurafenib + cetuximab or trametinib or cobimetinib
- Patient 3: trametinib or cobimetinib or sorafenib or regorafenib

**Conclusion:** Gene and Variant Analysis in context of each Patient’s Disease
Landscape of Solid Tumor Oncology Biomarker Testing

<table>
<thead>
<tr>
<th>Organ</th>
<th>Cancer</th>
<th>Biomarker</th>
<th>Assay</th>
<th>Drug</th>
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<tbody>
<tr>
<td>Breast</td>
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<tr>
<td>Lung</td>
<td>NSCLC</td>
<td>EGFR, ALK, ROS1, BRAF, PDL1, KRAS</td>
<td>Sequencing, FISH (IHC), Sequencing, IHC</td>
<td>EGFR: Erlotinib, gefitinib, afatinib, ALK: Crizotinib, certinib, ROS1: Crizotinib, BRAF: Dabrafenib-trametinib, PDL1: Pembrolizumab</td>
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<td>RET, BRAF, EGFR, HER2, KRAS, ALK, MET, ROS1</td>
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<td>BRAF</td>
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<td>BRAF: Vemurafenib, dabrafenib</td>
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- Many biomarkers show mutually exclusive genetic alterations.
- Tests usually performed sequentially/individually and therefore increases turnaround time to yield final results.
- Uses many slides instead of one or just a few.
### NGS is the Solution to Reduce TAT and Sample Size

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All these genetic alterations except for PDL1 can be tested by a single NGS assay with mutations, fusions and copy number variations, e.g., Oncomine Focus Assay (OFA)
We Offer 4 Panels for Solid Tumor Tests

- Lung cancer (24 genes)
- Colorectal cancer (6 genes)
- Melanoma (11 genes)
- Complete tumor (52 genes)

*Italic*: Not members of OFA for that aberration class

**DNA Panel**

| AKT1 | ALK | AR | BRAF | CDK4 | CTNNB1 | DDR2 | EGFR | ERBB2 | ERBB3 | ERBB4 | ESR1 | FGFR2 | FGFR3 | GNA11 | GNAQ | HRAS | IDH1 |
|------|-----|----|------|------|--------|------|------|-------|-------|-------|------|-------|-------|-------|------|------|------|------|
| IDH2 | JAK1| JAK2| JAK3 | KIT  | KRAS   | MAP2K1| MAP2K2| MET   | MTOR  | NRAS  | PDGFRA| PIK3CA| PIK3CA| RAF1  | RET  | ROS1 | SMO  |

**Copy Number Variants, n=19**

<table>
<thead>
<tr>
<th>ALK</th>
<th>FGFR3</th>
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<tr>
<td>AR</td>
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<td>MET</td>
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<td>CDK6</td>
<td>MYC</td>
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<td>MYCN</td>
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<td>ERBB2</td>
<td>PDGFRA</td>
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<tr>
<td>FGFR1</td>
<td>PIK3CA</td>
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<td>FGFR2</td>
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**Fusion drivers, n=23**

<table>
<thead>
<tr>
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<th>FGFR2</th>
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<tbody>
<tr>
<td>AKT3</td>
<td>FGFR3</td>
</tr>
<tr>
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<td>MET</td>
</tr>
<tr>
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<td>NTRK1</td>
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<tr>
<td>ETV1</td>
<td>PDGFRA</td>
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<tr>
<td>ETV4</td>
<td>PPARG</td>
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<td>ETV5</td>
<td>RAF1</td>
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<tr>
<td>ERBB2</td>
<td>ROS1</td>
</tr>
<tr>
<td>FGFR1</td>
<td></td>
</tr>
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</table>

Note: 52 genes total
# Landscape of Current Myeloid Biomarker Testing

<table>
<thead>
<tr>
<th>Disease</th>
<th>Biomarker</th>
<th>Assay</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>FLT3</td>
<td>PCR, NGS</td>
<td>Midostaurin (Rydapt)</td>
</tr>
<tr>
<td></td>
<td>IDH2</td>
<td>PCR, NGS</td>
<td>Enasidenib (Idhifa)</td>
</tr>
<tr>
<td>CML</td>
<td>BCR/ABL1</td>
<td>PCR, NGS</td>
<td>Imatinib (Gleevec)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dasatinib (Sprycel)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Nilotinib (Tasigna)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>Bosutinib (Bosulif)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Ponatinib (Iclusig)</td>
</tr>
<tr>
<td>APL</td>
<td>t(15;17)(q22;q12)</td>
<td>PCR, FISH, karyotype, NGS</td>
<td>ATRA</td>
</tr>
<tr>
<td></td>
<td>/PML-RARA</td>
<td></td>
<td>Arsenic trioxide</td>
</tr>
<tr>
<td>MPN</td>
<td>JAK2</td>
<td>PCR, NGS</td>
<td>Ruxolitinib</td>
</tr>
</tbody>
</table>

- NGS is the solution to reduce No. of Assays, TAT and sample size
We Offer 3 Panels for Heme NGS Tests

- AML (55 genes)
- MPN (38 genes)
- Heme (74 genes)
Why Choose Oncomine Assays?

- **Platform**: Ion Torrent S5/Chef
- **Panel**: all genes with actionable mutations and fusions
- **Small sample size**: 10ng DNA/RNA from FFPE, blood or bone marrow
- **Shorter TAT**: Tumor 5-7 days; heme 3-5 days
- **Single Workflow**: process DNA/RNA sequencing simultaneously with automation workflow
- **Reporting**: Oncomine Knowledgebase Reporter
Summary of NGS Assays

- NGS has become the standard for molecular assays and harbors enormous potential value to the practice of oncology.

- Oncomine NGS assay was chosen because of low sample input, improved TAT, intelligent NGS content design, single workflow, and proven clinical utility.

- Guidelines for comprehensive biomarker analysis in solid tumors and hematological disorders are available.

- NGS results will change the paradigm of cancer therapy and facilitate precision medicine.
Case 1

- This 49 year old woman was transferred on 2/9/19 with WBC of 57,900, a Hgb of 5.3 and plts of 15K. Her \textsuperscript{O}_2 saturation was 97.7% on nasal \textsuperscript{O}_2 suppl.

- Her peripheral blood smear differential revealed 71% blasts/promonocytes, 8% neutrophils, a Hgb of 5.3 and platelets of 15K. Normal counts were last reported on 6/25/18.

- SOB developed after 1 transfusion and chest X-ray disclosed diffuse bilateral infiltrates on 2/9/2019; hypotension requiring pressor support ensued.

- BAL, bronchial biopsy and cultures were negative for evidence of infection.
Case 1 - peripheral blood
Case 1 - peripheral blood
Case 1 - peripheral blood
Case 1 - peripheral blood
Case 1 - Chest X-ray - 2/5/19
Hospital Course

WBC increased to 100K on 2/12. Initially treated with hydroxyurea, pending NGS analysis of peripheral blood, as she was too ill to undergo a bone marrow biopsy.

Respiratory status worsened with increasing pulmonary infiltrates, resulting in intubation and ventilator support.
NGS analysis resulted an AML defining mutation [inv 16/ CBFB/MYH11 fusion]; typical of acute myelomonocytic leukemia (old FAB M4).

An additional mutation in the PTPN11 gene was also found with a 11% allele frequency (recently reported as adverse if >40%).

Negative for NPM1, CEBPA, FLT3 ITD/ TDK, and IDH1/2 mutations.

Induction chemotherapy was initiated on 2/17. Respiratory status improved and extubated.
NGS AML Molecular Profile

• 55 genes

• Hotspot genes: FLT3, CSF3R, DNMT3A, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, PTPN11, RUNX1, SF3B1, SRSF2, U2AF1, WT1

• Full genes: ASXL1, BCOR, CEBPA, EZH2, PHF6, STAG2, TET2, ZRSR2

• Fusion drivers: ABL1, ALK, BCL2, BRAF, CCND1, CREBBP, EGFR, ETV6, FGFR1, FGFR2, FUS, HMGA2, JAK2, KMT2A, MECOM, MET, MLLT10, MLLT3, MYBL1, MYH11, NTRK3, NUP214, PDGFRB, PDGFRA, RARA, RBM15, RUNX1, TCF3, TFE3

• Expression genes: BAALC, MECOM, MYC, SMC1A, WT1
Case 1: Hospital Course

WBC responded to nadir of 0.1 on 2/22 but did not recover through remainder of course.

Increased respiratory distress developed requiring re-intubation, pulmonary hemorrhage and by gram negative septic shock (Acinetobacter baumannii from BAL). She unfortunately expired on 3/7/19.
Case 1 - peripheral blood post induction
CXR: 2/21/19

CXR: 3/2/19
Case 1: Take home points

- NGS for hematopoietic neoplasms may be performed on peripheral blood, marrow aspirate or clot sections.

- May detect mutations/ rearrangements and copy number variations, replacing some FISH and PCR based analyses.

- Turnaround equal to or more rapid than older assays and chromosome analysis.

- FISH still required/ desirable for monosomies, trisomies, large deletions (i.e. 5, 7), rapid detection of PML/RARA, other rearrangements.
Substrates for NGS analysis

- Peripheral blood or bone marrow aspirate
- Aspirate (marrow or any site) smears, including archived smears
- Formalin fixed, paraffin embedded, non-decalcified tissues (most common single source)
- Certain platforms can perform single or multiple gene analyses for solid tumors using circulating tumor cells/ DNA, i.e. T790M EGFR mutation.
Case 2: Clinical History

This 65 year old Vietnamese man presented in late June, 2015 with shortness of breath. A right pleural effusion and right middle lobe mass were discovered. Both were positive for adenocarcinoma.

CT scan of the head was negative for metastatic disease.

The biopsy tissue was submitted for single assay molecular analyses.

**EGFR L858R exon 21 mutation identified.**

ALK rearrangement negative.

Following the results, he was begun on single agent adjuvant therapy with erlotinib (Tarceva).
Case 2: imaging 6/29/2015

CT Thorax W/ Contrast
5MM STD SS40
6/29/2015 2:06:48 PM
30144691
ISOVUE 300 70mls
LOC: -168.50
THK: 5
FFS

RD: 360
Tilt: 0
mA: 121
KVP: 120
Acq no: 1

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Z: 1
C: 40
W: 495
DFOV:36x36cm
Compressed 8:1
IM: 35 SE: 3
Case 2: 6/30/2015- post pleural drainage
Case 2: CT guided biopsy
Case 2: CT guided biopsy
Case 2: TTF-1/ Napsin A
Case 2: pleural fluid

Napsin A

TTF-1
Stable RUL nodule/ disease through 6/2016.

Radiation Rx administered to RUL and RML disease in Feb. and March of 2017.

No new disease through January 2018
In 7/2018, bilateral lung nodules were discovered, the largest 1.5 cm in the right lower lobe.

Biopsy and repeat molecular analysis were performed, now using the NGS Targeted Lung Profile.
Case 2: imaging 7/2018 - scarring RUL
Case 2: imaging 9/7/2018 - new lung nodules
Case 2: CT-Lung biopsy - 9/7/2018
NGS Target Gene Panel Lung Cancer

- **24 genes:**

- **Hotspot:** AKT1, ALK, BRAF, CDK4, DDR2, EGFR, ERBB2, FGFR1, FGFR2, FGFR3, HRAS, JAK2, KRAS, MAP2K1, MET, MYC, NRAS, PDGFRA, RET, ROS1

- **Copy Number Variation (CNV):** CCND1, FGFR1, MET

- **Fusion:** ALK, MET, NTRK1, NTRK2, NTRK3, RET, ROS1
Case 2: Clinical History (continued)

NGS testing disclosed the same exon 21 L858R mutation.

EGFR T790M mutation was also detected, conferring resistance to erlotinib, afatinib or gefinitib (EU) therapy.

Options for additional treatment: osimertinib (3rd generation EGFR TKI) or afatinib plus cetuximab.

Placed on osimertinib therapy with stable current stable disease.
Acquired Resistance to TKI

- EGFR mutation positive tumors respond well to tyrosine kinase inhibitors (TKI’s).

- The vast majority eventually develop resistance, 50-60% are due to secondary EGFR mutation T790M.

- Other mutations and transformation (small cell) account for another 20-25%; 15-20% unexplained.

- C797S mutation described for resistance to osimertinib.
Case 2: Take home points

- Re-testing of new localized or distant metastatic disease by NGS allows for comprehensive analysis for detection of new mutations such as EGFR T790M or C797S.

- The presence of EGFR T790M allows for changing therapy to osimertinib with likelihood of response.

- Resistance mutations continue to evolve with each generation of TKI.

- Similar approaches may be taken for kit mutations in the treatment of GIST or progression mutations in hematologic neoplasms.
Case 3

• This 49 year old woman presents with a 2 month history of a painful lump over the left eye.

• She awoke on the morning of admission with left eyelid and cheek bone swelling and dizziness.

• Examination revealed left frontal and maxillary swelling.

• CT and MRI disclosed a 3-4 cm mass involving the frontal bone and adjacent soft tissues. A partial resection was undertaken by neurosurgery.
Case 3 MRI - Axial T1
Case 3 MRI - post contrast
Case 3 - Diagnosis

- Malignant melanoma, likely metastatic.
- Melanoma NGS analysis revealed BRAF V600K and V600M mutations.
- BRAF V600E mutation analysis by IHC (VE1 antibody) was negative.

BRAF mutated
Thyroid Papillary ca
NGS Target Gene Panel Melanoma

• 11 genes:
  
• BRAF (40-50%, most common in cutaneous)
• NRAS (13%, 15-20% of cutaneous)
• MEK (6%); CTNNB1 (2-3%)
• KIT: 2-3% but in 15-40% of acral melanomas
• GNA11 (2%) and GNAQ (1%): 80% of uveal
• CCND1, CD4K, ERBB4, MAP2K1,
Take home points

Current treatment of metastatic melanoma:

- Nivolumab (anti-PD-1) + ipilimumab (anti-CTLA-4) (+/- PD-L1 analysis with Dako 28.8 assay for Nivolumab) or Pembrolizumab (no specific assay required).

- Vemurafenib/ dabrafenib: response predicted by BRAF V600E, M or K mutations. MEK inhibitors (trametinib and cobimetinib) also used.

- Dabrafenib + trametinib vs vemurafenib + cobimetinib

- V600E IHC is predictive of BRAF mutation if strongly immunoreactive; if negative, mutation testing is required.

- Kit mutated tumors may respond to imatinib.
Case 4: Pulmonary Carcinoma

- This 73 year old was diagnosed with pulmonary adenocarcinoma, gr2 in 2011. Initially treated by LL lobectomy, Stage T1b N1.

- 1-7-13 to 1-10-13 SBRT for R lung local recurrence.

- 12-08-15 right lung nodule biopsy

- 12-18-15 PET CT: progression of disease with numerous pulmonary nodules, no FDG avid disease below the diaphragm

- 1-25-16 MRI Brain: no evidence of malignancy
Case 4: Pulmonary Carcinoma (2)

1-29-16: Carboplatin and Pemetrexed 6 cycles (completed 5-13-16)

2-22-16 Outside molecular testing: negative for EGFR, EML4-ALK, KRAS mutations; Low PD-L1 (2-4%)

3-31-16 CT Chest: numerous bilateral metastases again seen, no lymphadenopathy, stable

2-23-17 CT Chest: interval increase in size of pulmonary nodules

3-07-17 MRI Brain: stable right parietal bone 1.2 cm enhancing osseous lesions, no intracranial metastases

3-10-17 changed to Nivolumab 240mg IV Q14
Case 4: Pulmonary Carcinoma (3)


- 11/27/17 CT chest: Progression in lungs when compared with previous CTs.

- 12/7/2017: R lung met biopsy, consistent with lung adenocarcinoma.
Case 4: 3/2015- bilateral nodules
Case 4: Progression - bilateral nodules
Case 4: Pathology- lung biopsy-1-2017
Case 4: Pathology - lung biopsy-1-2017
Case 4: Pathology - lung biopsy-1-2017
Case 4: Pathology - lung biopsy-1-2017

TTF-1/Napsin A

CK7
Case 4: Molecular analysis by NGS

- 1/22/2018: NGS showed EML4-ALK- translocation.

- Low PD-L1 (1-4%).

- Alectinib 600 mg bid was started.

- Stable disease through 1/2019.
Case 4: 1-2019- stable disease
Case 4: Take home points

- Targeted NGS for NSCLC detects the same gene fusions as FISH or PCR, with added sensitivity as compared to FISH (dependent on cell sampling, technical factors of the assay).

- Repeat testing with NGS covered a more broad panel than EGFR, ALK analysis from 3 years prior.

- Repeat testing with NGS uncovered the EML4-ALK fusion not detected by FISH at the time of primary diagnosis; allowing for directed single agent TKI therapeutic response.

Deans ZC, et al., Integration of next-generation sequencing in clinical diagnostic molecular pathology laboratories for analysis of solid tumours; an expert opinion on behalf of IQN Path ASBL. Virchows Arch. 2017 Jan;470(1):5-20.


Acknowledgements

• Our pathologist colleagues for their dedicated expertise in providing high-quality care for our patients and contributing case material.

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• We could not provide these vital services without you!