Next Generation Sequencing of Hematologic Neoplasms

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Learning Objectives:

1. List a few examples of the types of NGS tests

2. Describe the clinical utility of NGS technology in the context of testing of hematologic neoplasms
Outline

• NGS background
• Overview of types of clinical NGS tests
• NGS panels
• Single gene tests
  – Lymphoid clonality testing by NGS
  – BCR-ABL1 kinase domain sequencing
• Conclusions
Next Generation Sequencing (NGS)

Impact of NGS

Cost per Genome


$100M $10M $1M $100K $10K $1K

Moore's Law

NIH National Human Genome Research Institute

genome.gov/sequencingcosts
1st generation sequencing - Sanger sequencing

- utilizes chain terminating dideoxynucleotides
- slow and laborious, method has been relatively unchanged for ~30 years
- data = mixture of sequences
- sequence data can be reviewed manually
- poor sensitivity for detection of variants (~15-20%)
- relatively long contiguous sequence can be generated (>600bp)
NGS - also known as massively parallel sequencing

- parallel single molecule sequencing
- millions of small fragments of DNA are immobilized on a solid surface, amplified (copied), and sequenced simultaneously
- during sequencing a signal (light, pH change) is detected when a base is incorporated
- short contiguous sequences (reads) are generated
- reads are aligned to a reference sequence and analyzed
- analysis is computationally intense
Comparison of NGS applications

<table>
<thead>
<tr>
<th>NGS Application</th>
<th>Cost/Time</th>
<th>Sensitivity (depth of coverage)</th>
<th>Portion of genome sequenced (breadth of coverage)</th>
<th>Suitable for MRD detection?</th>
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</thead>
<tbody>
<tr>
<td>Whole genome sequencing</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>Whole exome sequencing</td>
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<tr>
<td>Mutation panels</td>
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<tr>
<td>Single gene tests</td>
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<td>++++</td>
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</table>
The power of NGS

- Study performed by the Cancer Genome Atlas Research Network
- 200 cases of de novo adult AML subjected to whole genome (50) or whole exome (15) sequencing
- Tier 1 – coding changes or splice sites
- Average of 13 overall (all tiers) mutations per case
- 23 genes significantly mutated (>5% of cases)
- Majority of cases demonstrated more than 1 clone based on distinct clusters of variant allele frequencies (VAFs)
Clinical impact of somatic mutations

- 738 patients with MDS, MDS-MPN
- 111 cancer associated genes were sequenced by NGS (gene panel)
- 78% of patients had 1 or more oncogenic mutations
- No systematic differences between DNA derived from bone marrow or peripheral blood

Higher overall number of oncogenic mutations correlated with worse outcome

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Clinically important information is derived from large scale genetic analysis by NGS:
The example of MDS

• *SF3B1* mutations are associated with favorable outcome

308 pts w/ myeloid neoplasms
MDS: 245
MDS/MPN: 34
AML-MDS: 29

111 gene mutation panel

*Almost all patients with RARS (refractory anemia with ring sideroblasts) had an SF3B1 mutation*
Clinical applications of NGS in hematology

- **Clinical applications:**
  - Whole genome sequencing (entire genome - ~3B base pairs)
  - Whole exome sequencing (~30M base pairs)
    - Sequencing limited to protein coding regions representing ~1% of genome
  - Mutation panels
    - Myeloid
      - AML prognostic markers – *FLT3, NPM1, CEBPA, ASXL1, IDH1/2*
      - Myelodysplastic syndromes (MDS) – cohesin and spliceosome genes frequently mutated
      - Myeloproliferative neoplasms (MPNs) – *JAK2, CALR, MPL, ASXL1*
      - Pan myeloid panels
    - Lymphoblastic leukemia and mature lymphoid neoplasms
      - Ph-like lymphoblastic leukemia
      - Diffuse large B cell lymphoma (BCR pathway mutations)
      - Mutations associated with T cell lymphoproliferative disorders (JAK-STAT pathway mutations)
      - Pan lymphoid panels
    - Congenital disorders – bone marrow failure syndromes, congenital hemolytic anemias
  - Detection of complex genomic abnormalities - copy number variants (CNVs) and translocations
  - Analysis of single genes with high complexity
    - Ex. lymphoid clonality and *IGH* or *TRG/TRB* genes
Whole genome sequencing

• Many of the biomarkers we now know to be important were discovered in whole genome sequencing studies (ie. *DNMT3A*, *IDH1/2*, etc)

• Not routinely performed in the clinical lab
  – Would need paired normal tissue for tumors
  – Time consuming
  – Expensive
  – Yields relatively low coverage (~30X) so results may be difficult to interpret, especially with low tumor burden

• Benefit: Not limited to selected targets
Spectrum of mutations in myeloid malignancies
AML, MDS, MPN and MDS/MPN overlap disorders

Cell signaling
- FLT3
- KIT
- JAK2
- MPL
- KRAS/NRAS
- PTPN11
- NF1
- CSF3R

Transcription
- CEBPA
- RUNX1
- GATA1/GATA2
- PHF6
- ETV6

Splicing
- SF3B1
- SRSF2
- ZRSR2
- U2AF1

Myeloid malignancies

Epigenetics
- DNMT3A
- TET2
- IDH1/IDH2
- ASXL1
- EZH2
- SUZ12
- KDM6A

Cell cycle
- TP53
- NPM1

Cohesin complex
- STAG2
- SMC1A
- SMC3
- RAD21

Matynia et al. et al. 2015. Archives of Pathology and Laboratory Medicine.
There is often a complex subclonal architecture in myeloid malignancies

Pre diagnosis
Ex. clonal hematopoiesis of uncertain significance (CHIP)

Diagnosis

Relapse

Matynia et al et al. 2015. Archives of Pathology and Laboratory Medicine.
# Variant Associations

<table>
<thead>
<tr>
<th>Gene</th>
<th>MPN</th>
<th>MDS</th>
<th>MDS/MPN</th>
<th>De novo AML</th>
<th>Secondary AML</th>
<th>Effect *</th>
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<td></td>
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</table>

From: Tietz textbook of Clinical Chemistry and Molecular Diagnostics, 6th Edition
Mutation panels: Variant reporting

- **Tiered strategy**
  
  - *A variety of systems are in use and this area currently lacks a uniform standard*

  - Higher tiers – more likely to be pathogenic or actionable

  - Variants of unknown significance (VUSs)

  - Lower tiers – less likely to be pathogenic or likely or known germline polymorphism

*NRAS* c.37G>C, p.Gly13Arg
*TET2* c.5284A>G, p.Ile1762Val
Clinical Scenario #1

• 52 year-old female presented with easy bruising and fatigue
  – CBC: WBC – 33 K/uL, Hgb – 9.6 g/dL, Platelets – 12,000 K/uL
  – Flow cytometry on BM aspirate: large CD34 negative atypical myeloid blast population (48% of leukocytes)
  – BM morphology – Acute myeloid leukemia
  – Cytogenetics/FISH – normal karyotype
Clinical scenario #1 - mutations

Mutation panel testing by NGS:

Tier 1 variants:

1. **NPM1 c.860_863dup, p.Trp288fs**
   - Variant frequency 35.5%
   - Associated with good prognosis except when a FLT3-internal tandem duplication mutation is present.

2. **FLT3 c.1802_1803ins45, p.Leu601_Lys602ins15**
   - Variant frequency 30.0%
   - Associated with early relapse and poor overall survival.

3. **DNMT3A c.2645G>A, p.Arg882His**
   - Variant frequency 41.2%
   - Commonly seen with NPM1 mutations in patients with CN-AML
   - DNMT3A R882 mutations are associated with poor outcome when compared to NPM1 mutated AML patients without DNMT3A mutations

**Conclusion – Poor prognosis; patient should proceed to BM transplant**
Clinical scenario #2

• 75 y/o male with complaint of fatigue and history of primary myelofibrosis

• CBC:
  – WBC: 40.05 k/uL
  – Hgb: 14.9 g/dL
  – MCV: 76.5 fL
  – Plts: 205 k/uL

• Cytogenetics: 46, XY, inv(12)
Clinical scenario #2 - Mutations

1. JAK2 c.1849G>T, p.Val617Phe
   - Variant frequency: 92.4%  Dominant clone, VAF implies LOH @ 9p
2. NRAS c.37G>C, p.Gly13Arg
   - Variant frequency: 16.5%
3. NRAS c.183A>C, p.Gln61His
   - Variant frequency: 8.6%  Subclone(s) implied by VAFs
4. ASXL1 c.2275_2284del, p.Gln760fs
   - Variant frequency: 8.3%

Variant frequencies illustrate complex underlying clonal architecture
Clinical scenario #3

- **Ph-like lymphoblastic leukemia**
  - Gene expression profile similar to Ph+ (BCR-ABL1+) lymphoblastic leukemia but do not have t(9;22);BCR-ABL1.
  - Affects children (10% with standard risk ALL) and adults (~20%).
  - Variety of molecular abnormalities that activate tyrosine kinase signaling pathways including rearrangements (CRLF2, ABL1, ABL2, etc) as well as mutations involving FLT3, IL7R, SH2B3, etc)
  - Worse outcome compared to non-Ph-like ALL
  - Benefit from tyrosine kinase inhibitor therapy
  - A properly designed NGS panel can assess for all of the potential molecular genetic abnormalities using a single test

Roberts KG et al 2016 J Clin Oncol
Ph-like lymphoblastic leukemia

Targetable kinase activating abnormalities in Ph-like ALL

Table 1. Kinase Fusions Identified in Ph-like Acute Lymphoblastic Leukemia.

<table>
<thead>
<tr>
<th>Kinase Gene</th>
<th>Tyrosine Kinase Inhibitor</th>
<th>Fusion Partners</th>
<th>Patients</th>
<th>5’ Genes</th>
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<tbody>
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<td>ABL1</td>
<td>Dasatinib</td>
<td>6</td>
<td>14</td>
<td>ETV6,11 NUP214,11 RCSD1,11 RANBP2,11 SNX2,12 ZMIZ112</td>
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<tr>
<td>ABL2</td>
<td>Dasatinib</td>
<td>3</td>
<td>7</td>
<td>PAG1,* RCSD1,* ZC3HAV1*</td>
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<td>4</td>
<td>SSBP2*</td>
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<tr>
<td>PDGFRB</td>
<td>Dasatinib</td>
<td>4</td>
<td>11</td>
<td>EBF1,11-13 SSBP2,* TNIP1,* ZEB2*</td>
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<td>CRFL2</td>
<td>JAK2 inhibitor</td>
<td>2</td>
<td>30</td>
<td>IGH,21 P2RY822</td>
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<td>JAK2</td>
<td>JAK2 inhibitor</td>
<td>10</td>
<td>19</td>
<td>ATF7IP,* BCR,11 EBF1,* ETV6,23 PAX5,11 PPFIBP1,* SSBP2,24 STRN3,11 TERF2,* TPR*</td>
</tr>
<tr>
<td>EPOR</td>
<td>JAK2 inhibitor</td>
<td>2</td>
<td>9</td>
<td>IGH,11 IGK*</td>
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<tr>
<td>DGKH</td>
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<td>1</td>
<td>ZFAND3*</td>
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<tr>
<td>IL2RB</td>
<td>JAK1 inhibitor, JAK3 inhibitor, or both</td>
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<td>1</td>
<td>MYH9*</td>
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<tr>
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<td>1</td>
<td>ETV6 25-27†</td>
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<td>1</td>
<td>KDM6A,* STAG2*</td>
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<td>IQGAP2*</td>
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<tr>
<td>TYK2</td>
<td>TYK2 inhibitor</td>
<td>1</td>
<td>1</td>
<td>MYB*</td>
</tr>
</tbody>
</table>

* The gene is a previously unreported fusion partner.
† ETV6–NTRK3 has been reported in multiple cancers, including congenital fibrosarcoma25,26 and secretory breast carcinoma,27 but it has not previously been described in acute lymphoblastic leukemia.26,27

Clinical scenario #3

• 29 year old male with relapsed B-lymphoblastic leukemia
  – Initial diagnosis 2012
    • Negative for t(9;22);BCR-ABL1
  – Tested at relapse using a single NGS panel (Foundation Medicine)
    • IGH-CRLF2 rearrangement
    • IKZF1 deletion
    • PAX5 missense mutation
  – Findings indicate “Ph-like” ALL
  – Awaiting transplant, may benefit from kinase inhibitor therapy
Panel-based NGS testing

Mutation panel testing by NGS

Pros
1. Variants are reported together, at the same time, on a single report
2. Interpretation takes into account all variants identified
3. Cost is less compared to multiple single gene tests
4. Variant frequencies provide information on subclonal structure
5. Pattern and identity of mutations facilitates accurate subclassification and prognostication
6. Detection of certain variants allows for the use of targeted therapies

Cons
1. May not be reimbursed by payers
2. Variants of unknown significance – what to do?
3. Some of the information is not currently actionable
Conclusions

• NGS is revolutionizing pathology and laboratory medicine

• Allows for true personalized medicine

• Facilitates use of targeted therapeutic strategies

• Costs are rapidly decreasing while the technology continues to improve

• Challenges remain
  – Cost and reimbursement
  – Data analysis
  – Variant interpretation
  – Other aspects of testing (ie. PCR) can affect the results!

• Today – panels and genetically complex single gene analysis; detection of targeted structural variants

• Future – routine comprehensive whole genome analysis of tumors