Next Generation Sequencing: Challenges and Strategies in Testing Patients with Circulating Hematopoietic Malignancies

Heather Zierhut, PhD, MS
Anne Deucher, MD, PhD
Federico Monzon, MD

34th Annual Education Conference
October 21-24, 2015 | David L. Lawrence Convention Center | Pittsburgh, PA
Learning Objectives:

1. Identify genetic syndromes that give rise to hematopoietic malignancies

2. Recognize complications that may arise when performing germline genetic testing in the background of hematologic malignancies

3. Understand the technical issues and clinical utility of mosaic results with NGS testing

4. Formulate and share questions related to specific cases discussed during the presentation
HEMATOPOIESIS

- Red Blood Cells
- Lymphocyte
- Monocyte
- Eosinophil
- Basophil
- Neutrophil

- White Blood Cells

- Platelets

- Marrow
Acute Leukemia
Acute Leukemia

- >20% circulating blasts (early precursors)

- Treated aggressively (immediate induction chemotherapy)

- Recommended that inherited disease testing be postponed until after the patient has been treated for the acute leukemia

- Important however to correlate any inherited disease testing subsequently performed with a complete blood count to ensure that testing was not performed during a possible relapse of disease
Chronic Circulating Hematopoietic Neoplasms

1) Lymphoid

   a) B-cell
      - chronic lymphocytic leukemia
      - follicular lymphoma
      - marginal lymphoma
      - mantle lymphoma
      - hairy cell lymphoma
      - lymphoplasmacytic lymphoma

   b) T cell

      - large granular lymphocytic leukemia
      - chronic NK-cell leukemia
      - adult T-cell leukemia/lymphoma
      - Sezary syndrome
      - T-cell prolymphocytic leukemia (usually aggressive but rarely can be chronic).
Chronic Circulating Hematopoietic Neoplasms

1) Lymphoid

- By morphology, these cells will resemble atypical mature lymphocytes and most hematology labs will place them in the lymphocyte category.

- Flow cytometry should be able to differentiate the normal B- and T-cells from the neoplastic cells, therefore for a definitive assessment of the percent of neoplastic disease present in the peripheral blood a flow cytometry assay report may be useful.

- Disease burden can change over time, however because the diseases are chronic diseases the change will likely be less dramatic (mantle cell lymphoma excluded).

- Counts obtained up to several weeks prior, as long as no therapy had been administered, should give an approximate representation of the percent burden of disease in the peripheral blood.
 Chronic Circulating Hematopoietic Neoplasms

2) **Myeloid**

  =(In peripheral blood) Neutrophils, eosinophils, basophils, monocytes

a) **Myelodysplastic syndrome (MDS)**

  - Cytopenias (low counts)
  - Dysplasia

b) **Myeloproliferative neoplasms (MPN)**

  e.g. chronic myeloid leukemia, essential thrombocytosis, polycythemia vera, primary myelofibrosis

  - Cytoses (high counts- red cell, white cell, and/or platelets)

c) **MDS/MPN neoplasms**

  e.g. chronic myelomonocytic leukemia, atypical CML, juvenille myelomonocytic leukemia (JMML)
Chronic Circulating Hematopoietic Neoplasms

2) Myeloid

- Flow cytometry is not usually useful in quantifying the percent peripheral blood involvement by the myeloid

- Sometimes ancillary tests can help quantify disease burden eg. (BCR-ABL RT-PCR in CML or FISH in MDS)

- Counts obtained up to several weeks prior, as long as no therapy had been administered, should give an approximate representation of the percent burden of disease in the peripheral blood
Learning Objective 1- Identify genetic syndromes that give risk to hematopoietic malignancies

Heather Zierhut, PhD, MS
University of Minnesota
Genetic causes of hematological malignancies

- Syndromic - increased hematologic risk is a component of the condition
- Hematologic risk is the major or only presentation of the condition
Typically categorized by the underlying molecular defect or the clinical presentation

**Suggested Reviews**


<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s)</th>
<th>Inheritance</th>
<th>Clues</th>
<th>Leukemia Type</th>
<th>Leukemia Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li-Fraumeni Syndrome</td>
<td>TP53</td>
<td>AD</td>
<td>Family hx malignancy - breast, soft tissue, brain, adrenal gland, bone</td>
<td>ALL, MDS, AML</td>
<td>1-3%</td>
</tr>
<tr>
<td>Biallelic Mismatch Repair Syndrome</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>AR</td>
<td>Tumors of diverse organ systems, including colorectal cancers and brain tumors in childhood</td>
<td>ALL, AML</td>
<td>Unknown, but high</td>
</tr>
</tbody>
</table>
**RECQ helicase disorders**

**Werner Syndrome**
- Normal development in the first decade of life
- Premature aging
- Loss/graying of hair
- Scleroderma-like skin
- Cataracts
- Diabetes
- Osteopetrosis in 30s
- Myocardial infarction and cancer by age 48
- **Increased risk of OS**

**Bloom Syndrome**
- Growth retardation
- Sun-sensitive erythematous skin lesion
- Recurrent infections
  - Otitis media
  - Pneumonia
- Chronic pulmonary disease
- Diabetes mellitus
- Learning disabilities
- Infertility issues
- **Increased risk cancer**

**Rothmund-Thompson**
- Poikiloderma
- Sparse hair/eyelashes
- Small Stature
- Skeletal/Dental abnormal
- Cataracts
- Increased risk of cancer
- **Especially high risk OS**

Photos obtained on e-Medicine
## DNA REPAIR

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s)</th>
<th>Inheritance</th>
<th>Clues</th>
<th>Leukemia Type</th>
<th>Leukemia Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fanconi Anemia</strong></td>
<td>FANCA-C, FANCD1-2, FANCE-G, FANCI-J, FANCL+</td>
<td>AR, XLR</td>
<td>Short stature; abnormal skin pigmentation; malformations of the thumbs, forearms, and more, bone marrow failure, increased risk of malignancy</td>
<td>MDS/AML, ALL</td>
<td>7% MDS; 9% AML 500-fold AML*</td>
</tr>
<tr>
<td><strong>Ataxia Telangiectasia</strong></td>
<td>ATM</td>
<td>AR</td>
<td>Progressive cerebellar ataxia (~1-4 yrs), oculomotor apraxia, choreoathetosis, telangiectasias of the conjunctivae, immunodeficiency, frequent infections, and an increased risk for malignancy</td>
<td>ALL</td>
<td>70-fold leukemia</td>
</tr>
<tr>
<td><strong>Nijmegen breakage syndrome</strong></td>
<td>NBS1</td>
<td>AR</td>
<td>Progressive microcephaly, IUGR, short stature, recurrent sinopulmonary infections, increased risk for cancer, POF in females.</td>
<td>ALL</td>
<td>Unclear</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Gene(s)</td>
<td>Inheritance</td>
<td>Clues</td>
<td>Leukemia Type</td>
<td>Leukemia Risk</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------------</td>
<td>-------------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Noonan syndrome</td>
<td>PTPN11, SOS1, KRAS, NRAS, RAF1, BRAF, SHOC2, MEPK1</td>
<td>AD</td>
<td>Short stature, facial dysmorphisms and congenital heart defects</td>
<td>TMD, JMML, CMML, ALL</td>
<td>Unknown, but high</td>
</tr>
<tr>
<td>CBL syndrome</td>
<td>CBL</td>
<td>AD</td>
<td>Noonan-like, highly variable</td>
<td>JMML</td>
<td>Unknown</td>
</tr>
<tr>
<td>Neurofibromatosis Type 1</td>
<td>NF1</td>
<td>AD</td>
<td>Multiple café-au-lait spots, axillary and inguinal freckling, multiple cutaneous neurofibromas, and iris Lisch nodules, learning disabilities</td>
<td>CMML/JMML, AML</td>
<td>11% MDS 200 to 500-fold JMML</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Gene(s)</td>
<td>Inheritance</td>
<td>Clues</td>
<td>Leukemia Type</td>
<td>Leukemia Risk</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>---------------------</td>
<td>-------------</td>
<td>----------------------------------------------------------------------</td>
<td>--------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Diamond-Blackfan Anemia</td>
<td>RPS19, RPS24, RPS17, RPL35A, RPL5, RPL11, RPS7, RPS26, RPS10, GATA1</td>
<td>Sporadic, AD, AR, XLR</td>
<td>Red blood cell aplasia, growth retardation, congenital malformations: craniofacial, upper-limb, heart, and genitourinary malformations</td>
<td>MDS/AML, ALL</td>
<td>5%</td>
</tr>
<tr>
<td>Shwachman-Diamond Syndrome</td>
<td>SBDS</td>
<td>AR</td>
<td>Pancreatic dysfunction and growth failure; and bone abnormalities, short stature and recurrent infections (neutropenia)</td>
<td>MDS/AML, ALL</td>
<td>5-24%</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Gene(s)</td>
<td>Inheritance</td>
<td>Clues</td>
<td>Leukemia Type</td>
<td>Leukemia Risk</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>------------------</td>
<td>-------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Amegakaryocytic thrombocytopenia</td>
<td>MPL</td>
<td>AR</td>
<td>Isolated thrombocytopenia and megakaryocytopenia with no physical anomalies, 1/22,500 in Jewish pt’s</td>
<td>MDS/AML</td>
<td>Unknown, rare reports</td>
</tr>
<tr>
<td>Thrombocytopenia and absent radii</td>
<td>RBM8A Del 1q21.1</td>
<td>AR, Sporadic</td>
<td>Short stature and skeletal abnormalities, underdevelopment of bones in the arms and legs, malformations of the heart and kidneys. facial features: micrognathia; prominent forehead; and low-set ears.</td>
<td>MDS/AML</td>
<td>Unknown, rare reports</td>
</tr>
<tr>
<td>Severe congenital neutropenia</td>
<td>ELANE, G6P C3, GFI1, HAX1, CSF3 R</td>
<td>AD, AR, X-linked</td>
<td>Neutropenia, recurrent fever, skin and oropharyngeal inflammation (i.e., mouth ulcers, gingivitis, sinusitis, and pharyngitis), and cervical adenopathy.</td>
<td>MDS/AML</td>
<td>8 to 25%</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Gene(s)</td>
<td>Inheritance</td>
<td>Clues</td>
<td>Leukemia Type</td>
<td>Leukemia Risk</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>--------------------------</td>
<td>-------------</td>
<td>------------------------------------------------------------------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Telomere Disorders &amp; Dyskeratosis congenita</td>
<td><em>CTC1, DKC1, TERC, TERT, TINF2, NOP10, NHP2, WRAP53</em></td>
<td>XL, AD, AR</td>
<td>DC - characterized by the triad of reticulated skin hyperpigmentation, nail dystrophy, and oral leukoplakia. Other telomeres - pulmonary fibrosis anticipation</td>
<td>MDS/AML</td>
<td>3%-33%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s)</th>
<th>Inheritance</th>
<th>Clues</th>
<th>Leukemia Type</th>
<th>Leukemia Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wiskott-Aldrich Syndrome</td>
<td>WAS</td>
<td>XL</td>
<td>Eczema, thrombocytopenia, immune deficiency, proneness to infection, and bloody diarrhea</td>
<td>ALL</td>
<td>2%</td>
</tr>
<tr>
<td>Bruton’s Agammaglobulinemia</td>
<td>BTK</td>
<td>XL</td>
<td>Immunodeficiency, proneness to infection</td>
<td>ALL</td>
<td>Unknown, rare</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Gene(s)</td>
<td>Leukemia Type</td>
<td>Leukemia Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>------------</td>
<td>---------------</td>
<td>-----------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial mosaic monosomy 7</td>
<td>Unknown</td>
<td>MDS/AML</td>
<td>Very high, early onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Down Syndrome</td>
<td>Trisomy 21</td>
<td>TMD, AML, ALL</td>
<td>10% TMD, 1-2% ALL-AML</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Genetic causes of hematological malignancies

- Syndrome where increased hematologic risk is a component to the condition
- Hematologic risk is the major or only presentation of the condition
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s)</th>
<th>Inheritance</th>
<th>Clues</th>
<th>Leukemia Type</th>
<th>Leukemia Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial AML</td>
<td><em>CEBPA</em></td>
<td>AD</td>
<td>Family history - MDS/AML</td>
<td>MDS/AML</td>
<td>Unknown, younger onset</td>
</tr>
<tr>
<td></td>
<td><em>DDX41</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial Platelet Disorder</td>
<td><em>RUNX1</em>, <em>ETV6</em>, <em>ANKRD26</em></td>
<td>AD</td>
<td>Thrombocytopenia, prolonged bleeding time, Family history – FPD/ MDS/AML</td>
<td>MDS/AML</td>
<td>Median: 35% AML; Young onset</td>
</tr>
<tr>
<td>Monocytopenia and Mycobacterial infection Syndrome</td>
<td><em>GATA2</em></td>
<td>AD</td>
<td>History of MAC infections, HPV infections, familial MDS/AML and/or lymphedema</td>
<td>MDS/AML</td>
<td>50%</td>
</tr>
<tr>
<td>Syndrome</td>
<td>Gene(s)</td>
<td>Inheritance</td>
<td>Clues</td>
<td>Leukemia Type</td>
<td>Leukemia Risk</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------</td>
<td>-------------</td>
<td>------------------------------------------------</td>
<td>---------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Familial PAX5 Syndrome</td>
<td><em>PAX5</em></td>
<td>AD</td>
<td>Family history - B-cell ALL</td>
<td>ALL</td>
<td>Unknown, but high</td>
</tr>
<tr>
<td>Familial SH2B3 Syndrome</td>
<td><em>SH2B3</em></td>
<td>AR</td>
<td>Autoimmune disorders, ALL, growth retardation, mild developmental delays</td>
<td>ALL</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Medical and Family History Questions

• Type of hematological malignancy

• Other clinical manifestations of the proband
  » Solid tumors (e.g. osteosarcoma)
  » Congenital anomalies (e.g. limb, growth)
  » Bone marrow failure or immunodeficiency
  » Bleeding (platelets) or infections (atypical, neutropenia)
  » Hallmark findings (premature aging, pulmonary fibrosis, skin pigmentation)

• Family history:
  » Same hematological or other malignancies
  » Multiple generation or several in one generation
  » Consider anticipation
Personal +/- Family history of MDS / AML

Family History of Solid Tumors: Li Fraumeni, +

Bone Marrow Failure: FA, DC, DBA, SD, TAR, AT, etc.

Family History of MDS/AML only = CEBPA, GATA2, Telomere disorder

Thromobocytopenia & Bleeding = FPD

Lymphodema, monocytopenia, & atypical infections: GATA2

Babushok et al., Best Practice & Research Clinical Haematology (2015) 28; 55-64.
Germline testing in the background of hematologic malignancy with recommendations regarding interpretation

Anne Deucher, MD PhD
Molecular Genetic Pathology
Hematopathology
Goals/Learning Objectives:

1. Describe limitations of germline genetic testing in patients with hematopoietic malignancies

2. Review illustrative case
Normal Blood

- Erythrocytes
- Neutrophil
- Lymphocyte
- Monocyte
- Platelets
Limitations of inherited disease testing in individuals with hematopoietic malignancies and suggested approaches to interpretation

1) **False positives**

   a) **Acquired mutations**

2) **False Negatives**

   a) Acquired *loss of the chromosomal region containing the germline mutation*

   *E.g. - In CLL, 55% of cases lack 13q*
   - BRCA2 is located on 13q12
   - Study has shown that BRCA2 deleted in 80% of CLL cases in acquired fashion when del 13q
2) **False Negatives**

b) **Acquired chromosome translocation/insertion/inversion** cause misaligning of NGS data.
1). Evaluate likelihood for false positive/false negative in genes of interest.

   a). Determine whether inherited gene of interest may be affected by hematopoietic neoplasm

<table>
<thead>
<tr>
<th>Frequent Cytogenetic Finding</th>
<th>Percent Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q deletion</td>
<td>55%</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>17%</td>
</tr>
<tr>
<td>11q deletion</td>
<td>17%</td>
</tr>
<tr>
<td>17p deletion</td>
<td>7%</td>
</tr>
</tbody>
</table>

* Specific hematopoietic neoplasms have recurrent cytogenetic and molecular changes
## Recurrent Cytogenetic Changes in MDS resulting in unbalanced changes

<table>
<thead>
<tr>
<th>Frequent Cytogenetic Finding</th>
<th>Percent Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ 8</td>
<td>10%</td>
</tr>
<tr>
<td>-7 or del(7q)</td>
<td>10%</td>
</tr>
<tr>
<td>-5 or del(5q)</td>
<td>10%</td>
</tr>
<tr>
<td>del(20q)</td>
<td>5-8%</td>
</tr>
<tr>
<td>-Y</td>
<td>5%</td>
</tr>
<tr>
<td>i(17q) or t(17p)</td>
<td>3-5%</td>
</tr>
<tr>
<td>-13 or del(13q)</td>
<td>3%</td>
</tr>
<tr>
<td>del(11q)</td>
<td>3%</td>
</tr>
<tr>
<td>del(12p) or t(12p)</td>
<td>3%</td>
</tr>
<tr>
<td>del(9q)</td>
<td>1-2%</td>
</tr>
<tr>
<td>Idic(X)(q13)</td>
<td>1-2%</td>
</tr>
</tbody>
</table>

1) Atlas of Genetics and Cytogenetics in Oncology and Hematology  
http://atlasgeneticsoncology.org/index.html

2) Cancer Genome Anatomy Project  
http://cgap.nci.nih.gov/Chromosomes/RecurrentAberrations
<table>
<thead>
<tr>
<th>Hereditary Cancer Syndrome Genes</th>
<th>Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>5q21</td>
</tr>
<tr>
<td>ATM</td>
<td>11q22-23</td>
</tr>
<tr>
<td>BMPR1A</td>
<td>10q22</td>
</tr>
<tr>
<td>BRCA1</td>
<td>17q21</td>
</tr>
<tr>
<td>BRCA2</td>
<td>13q12</td>
</tr>
<tr>
<td>BRIP1</td>
<td>17q22</td>
</tr>
<tr>
<td>CDH1</td>
<td>16q22</td>
</tr>
<tr>
<td>CDK4</td>
<td>12q14</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>9p21</td>
</tr>
<tr>
<td>CHEK2</td>
<td>22q12</td>
</tr>
<tr>
<td>EPCAM</td>
<td>2p21</td>
</tr>
<tr>
<td>MEN1</td>
<td>11q31</td>
</tr>
<tr>
<td>MET</td>
<td>7q31</td>
</tr>
<tr>
<td>MLH1</td>
<td>3p21</td>
</tr>
<tr>
<td>MSH2</td>
<td>2p21</td>
</tr>
<tr>
<td>MSH6</td>
<td>2p16</td>
</tr>
<tr>
<td>MUTYH</td>
<td>1q34</td>
</tr>
<tr>
<td>NBN</td>
<td>8q21</td>
</tr>
<tr>
<td>PALB2</td>
<td>16p12</td>
</tr>
<tr>
<td>PALLD</td>
<td>4q32</td>
</tr>
<tr>
<td>PMS2</td>
<td>7p22</td>
</tr>
<tr>
<td>PTCH1</td>
<td>9q22</td>
</tr>
<tr>
<td>PTEN</td>
<td>10q23</td>
</tr>
<tr>
<td>RAD51C</td>
<td>17q22</td>
</tr>
<tr>
<td>RET</td>
<td>10q11</td>
</tr>
<tr>
<td>SMAD4</td>
<td>18q21</td>
</tr>
<tr>
<td>STK11</td>
<td>19p13</td>
</tr>
<tr>
<td>TP53</td>
<td>17p13</td>
</tr>
<tr>
<td>VHL</td>
<td>3p25</td>
</tr>
</tbody>
</table>
b). Review available karyotype/FISH testing to determine whether patient’s neoplasm has gain/loss of chromosomal region/gene or translocation which may affect inherited disease testing

* Neoplasms can attain any acquired change, even if not recurrent
*** Can’t definitive rule out change/deletion based on Cytogenics/FISH results****

* Cytogenetics testing may not capture all changes due to:

1) a cryptic change for which a FISH probe was not tested,

2) technical aspects of karyotype assay (metaphase cells analyzed),

3) evolution of disease or emergence of a low level clone since the time of cytogenetic testing,

4) loss of heterozygosity in tumor which masks allele loss
2). Determine degree of involvement of background neoplasm

* Percent involvement can sometimes be obtained and sometimes can be difficult to ascertain

a) If lymphoid neoplasm, lymphocyte count in recent CBC can give upper limit of degree of involvement

b) For B-cell and sometimes T/NK cell neoplasms, flow cytometry can give more accurate degree of neoplastic cell involvement in peripheral blood

c) For myeloid disorders, percent of non-lymphoid white cells (neutrophils, monocytes, eosinophils, basophils) can give upper limit of degree of involvement but flow cytometry not useful.
d) Ancillary tests may be useful to determine degree involvement

1) FISH testing on unselected interphase cells for known acquired change - eg. T14;18 in follicular lymphoma (50/200 cells)

2) Quantitative molecular tests
   - eg. RT-PCR for BCR-ABL in CML

* Karyotype not useful as only detects abnormal metaphases
3). Determine degree of sensitivity to detection and reporting structure of mosaic changes in lab performing test

a) Sensitivity of detection dependent on:

1) Depth of sequencing (number of reads)

2) Lab’s bioinformatic algorithms

b) Reporting dependent on:

1) Assay validation

2) Detection threshold/policy set
4). Correlate with other findings

a) **SNPs** in gene can indicate **heterozygosity** of allele

- E.g. - BRCA2 benign SNP would indicate both alleles detectable

  - If SNP present in ~50/50 allele balance, would feel comfortable both alleles present in germline ratio

b) **Family studies** can conclude whether variant present is germline

- E.g. - If variant found in family member, can rule out as acquired

c) Correlate with testing on tumor/skin biopsy

- E.g. - If variant present in tumor in heterozygous/homozygous (secondary to LOH) fashion, would suggest germline

* Study shows that buccal swabs/mouthwash can rarely be significantly contaminated with peripheral blood (up to 70% of nucleated cells are WBC)
Illustrative case:

Hereditary cancer predisposition germline testing in patient with background of chronic lymphocytic leukemia
Chronic Lymphocytic Leukemia (CLL):

- Neoplastic disorder of B-cells in peripheral blood

- Incidence of 2-6 cases/100,000 people/ year

- When present only in tissues (more rare), called small lymphocytic lymphoma (SLL)

- Most patients are asymptomatic, but some present with cytopenias, splenomegaly, and/or lymphadenopathy

- Diagnosed using combination of CBC with differential, flow cytometry (morphology can support), and FISH/IPOX testing
**Routine Chronic Lymphocytic Leukemia (CLL) Prognostic Testing:**

1) **Karyotype testing:**

- Performed at diagnosis

- Clonal changes seen in 82% of the cases

- Certain recurrent cytogenetic abnormalities have good/bad prognosis
2) **FISH testing:**

- Performed at diagnosis to investigate most frequent cytogenetic prognostic markers

**Table I: Recurrent Cytogenetic Changes in CLL**

<table>
<thead>
<tr>
<th>Frequent Cytogenetic Finding</th>
<th>Percent Involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q deletion</td>
<td>55%</td>
</tr>
<tr>
<td>Trisomy 12</td>
<td>17%</td>
</tr>
<tr>
<td>11q deletion</td>
<td>17%</td>
</tr>
<tr>
<td>17p deletion</td>
<td>7%</td>
</tr>
</tbody>
</table>

* BRCA2 deleted 80% of patients with 13q deletion (~45% CLL)*
Non-Routine Chronic Lymphocytic Leukemia (CLL) Prognostic Testing:

1) **TP53 sequencing:**

- Smaller TP53 mutations seen in 8.5-15% of CLL cases
- TP53 mutations associated with worse outcome
Patient:

- 64 year old male with CLL

- CBC 1 week prior to NGS blood draw showed 60% lymphocytes

- Flow cytometry performed 3 weeks prior to NGS blood draw demonstrated 50% of lymphocytes were kappa-light chain restricted with immunophenotype consistent with CLL

  * So ~30% of cells are neoplastic CLL cells

- Mild anemia only, so no chemotherapy for CLL

- Patient also with personal and family history of colon and breast cancer

- Desired testing of germline for cancer predisposition genes:

  = APC, ATM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, MEN1, MET, MLH1, MSH2, MSH6, MUTYH, NBN, PALB2, PALLD, PMS2, PTCH1, PTEN, RAD51C, RET, SMAD4, STK11, TP53, VHL
**Results:**

1) Pathogenic TP53 point mutation in mosaic fashion.

2) BRCA2 copy number variation was normal
Interpretation:

1) Consider likelihood of false positive/false negative
   
   A) False positive= *TP53*
   
   B) False negative= *BRCA2* or other

2) Review patient’s disease related testing
   
   A) Karyotype= normal
   
   B) FISH= positive for 13q del in 64/200 cells (~30% of cells)
      
      * BRCA2 deleted 80% of patients with 13q deletion (~45% CLL)

3) Determine degree of involvement
   
   A) CBC= 60% lymphocytes
   
   B) Flow cytometry= 50% of lymphocytes CLL = 30%

4) Determine sensitivity of detection of mosaic findings
   
   = 10% by lab’s report
Results:

1) Pathogenic TP53 point mutation.

* Mutation present in 14% of alleles = consistent with heterozygous in CLL cells (30% of cells)

*Can’t rule out germline mosaicism or technical artifact

*Would suggest family testing/correlation with tumor/skin biopsy to confirm
Results:

2) BRCA2 copy number variation was normal

* Signal was below baseline but didn’t meet threshold for calling deletion

* Copy number variation detection is less sensitive for mosaic detection so can’t rule out BRCA2 del in subset of cells (~15% alleles)

* Take comfort that lab would detect germline pathogenic mutation even if BRCA2 deleted in CLL cells (41% of alleles would be mutant)

a) Several benign heterozygous SNPs with high frequency in population present in BCRA2 in patient

b) Skin biopsy/fibroblast culture could be performed if clinical necessary
Interpreting mosaic results with NGS testing: understanding how circulating somatic tumors differ from germline mosaic findings in peripheral blood samples

Federico A. Monzon, M.D.
Invitae
Agenda

• Understanding Mosaicism
• Understanding Mosaic NGS Results
• Cases
• Caveats and Implications for Counseling

• Learning objective #3: Understand the technical issues and clinical utility of mosaic results with NGS testing
Mosaicism is a common phenomenon

X Inactivation
Mosaicism

• Definition of “mosaic”
  » Dictionary: “derived from the presence of many different pieces to form a single whole”
  » Genetics: “presence in a single individual of two genetically distinct populations of cells that differ from each other at the level of DNA sequence but that derive from a single zygote

• A genetic change acquired post-fertilization
  » Constitutional (during embryogenesis)
  » Somatic (acquired later in life)
    • Occurs frequently
    • Can lead to pre-neoplastic>neoplastic changes
Chimerism: single organism composed of genetically distinct cells

A. Fusion
   - Cell lines: 46,XX(CA) 46,XY(DB)
   - Key:
     - X bearing sperm
     - Y bearing sperm
     - ovum
     - polar body
     - zona pellucida
   - Haploid genomes:
     - A, B, C, D
     - A¹ (polar body)

B. Fusion
   - Cell lines: 46,XX(CA) 46,XY(DA¹)

C. Fusion
   - Cell lines: 46,XX(CA) 46,XY(DA)

Martin Powers M.D.
Constitutional mosaicism

Somatic  

Gonosomal  

Germline

Biesecker LG, Spinner NB. Nat Rev Genet 2013 PMID: 23594909
Constitutional mosaicism
Timing is Everything

Campbell IM et al. Trends Genet 2015 PMID 25910407
Figure 2. Phenotypic manifestations of mosaic mutations. (A) Inflammatory nevus affecting the left side of the body of an individual aged 1 month with CHILD syndrome. Note the striking demarcation at the midline. Reproduced with permission from Chander et al. [7]. (B) Cerebriform connective tissue nevus on the plantar surface of the foot in an individual aged 11 years with Proteus syndrome. Reproduced with permission from Beachkofsky et al. [91]. (C) Axial T2-weighted image showing markedly enlarged left cerebral hemisphere in a newborn with hemimegalencephaly. Reproduced with permission from Lang et al. [92]. (D) Hyperpigmentation following lines of Blashko in an individual with linear and whorled nevoid hypermelanosis. Reproduced with permission from Molho-Pessach and Schaffer [93].
Reversion of inherited mutations

- Mosaicism due to reversions to normal of an inherited mutation has been reported
- Multiple disorders like Tyrosinemia, SCID, Fanconi Anemia and others
- Mechanisms include:
  - Intragenic recombination
  - Mitotic gene conversion
  - Second site compensating mutations
  - DNA slippage
  - Chromothripsis
  - Unknown

Hirschhorn R. J Med Genet 2003 PMID 14569115
McDermott DH et al. Cell 2015 PMID 25662009
Somatic mutations in cancer
Adenoma>Carcinoma Sequence

Schematic of the morphologic and molecular changes in the adenoma-carcinoma sequence
Somatic mutations in cancer and clonal evolution

Also present in solid tumors

Gerlinger, M. NEJM 2012 PMID 24487277
Mosaicism and NGS
Sanger (Dideoxy) DNA Sequencing

Single-stranded DNA to be sequenced

Sequencing reactions using different fluorescent-labeled primers with each dideoxynucleotide

ddG  ddA  ddC  ddT

Pool products
Electrophoresis

Fragment migration

Laser beam

Photomultiplier

Fluorescence

Computer

34th Annual Education Conference
October 21-24, 2015 | David L. Lawrence Convention Center | Pittsburgh, PA
Example of NGS sequence results

Voelkerding et al. J Mol Diagn 2010
Case #1 – Missed by Sanger

- 50-60 y/o male
- Colon Cancer in late 30’s
  - ~10 adenomas
  - Sebaceous neoplasms
  - Lipomas
- Family History
  - 2x 1st degree relatives with prostate cancer
  - Mother with leukemia
- Prior testing:
  - Normal IHC
  - MLH1/MSH2 wild type
Case #1 - Results

Total count: 1765
A: 435 (25%, 225+, 210-)
C: 1 (0%, 0+, 1-)
G: 1329 (75%, 666+, 663-)
T: 0

Reverse complementary sequence
Case #2
Follow up on Somatic Testing

- Jewish woman of mixed Ashkenazi (paternal) and Bulgarian (maternal)
- Invasive ductal TN breast cancer in her early 40s
- Family history
  - daughter with acute lymphoblastic leukemia in her teens
  - brother with a CNS tumour in his 40s
  - father with a malignant CNS tumour in his late 50s
  - MGF with a malignant tumour of unknown pathological features in his 70s
  - 2 maternal grand-aunts with breast cancer

Friedman E et al. Br J Cancer. 2015 PMID: 25633036
Somatic testing results

<table>
<thead>
<tr>
<th>PATIENT RESULTS</th>
<th>TUMOR TYPE: BREAST INVASIVE DUCTAL CARCINOMA (IDC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 genomic alterations</td>
<td>Genomic Alterations Identified†</td>
</tr>
<tr>
<td>1 therapy associated with potential clinical benefit</td>
<td>ALK amplification</td>
</tr>
<tr>
<td>0 therapies associated with lack of response</td>
<td>BRCA1 K652fs*21</td>
</tr>
<tr>
<td>9 clinical trials</td>
<td>MYC amplification</td>
</tr>
<tr>
<td></td>
<td>MYCN amplification</td>
</tr>
<tr>
<td></td>
<td>TP53 E258G</td>
</tr>
<tr>
<td></td>
<td>Additional Disease-relevant Genes with No Reportable</td>
</tr>
</tbody>
</table>

The protein encoded by BRCA1 is involved in the maintenance of genomic stability, including DNA repair, cell cycle checkpoint, and chromosome segregation (O'Donovan and Livingston, 2010; 20400477). BRCA1 K652fs*21 results in a premature truncation at codon 652 out of 1864, prior to both of the BRCT domains. This truncation leads to a loss in function, which is consistent with its roles in DNA repair and homologous recombination.

“BRCA1 K652fs*21 results in a premature truncation at codon 652”

“….in the appropriate clinical context, testing for the presence of germline mutations in BRCA1 is recommended.”
Germline Testing Results

Table 1. Mutational load in different tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Reads indicating insertion</th>
<th>Total depth at this position</th>
<th>% Reads carrying the insertion</th>
<th>% Heterozygous cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (draw 1, extraction 1)</td>
<td>164</td>
<td>3307</td>
<td>5.0</td>
<td>9.9</td>
</tr>
<tr>
<td>Blood (draw 1, extraction 2)</td>
<td>143</td>
<td>2889</td>
<td>4.9</td>
<td>9.9</td>
</tr>
<tr>
<td>Blood (draw 2)</td>
<td>127</td>
<td>2460</td>
<td>5.2</td>
<td>10.3</td>
</tr>
<tr>
<td>Buccal swab</td>
<td>149</td>
<td>2207</td>
<td>6.8</td>
<td>13.5</td>
</tr>
<tr>
<td>Normal breast tissue</td>
<td>–</td>
<td>–</td>
<td>5.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Breast tumour</td>
<td>–</td>
<td>–</td>
<td>47.0</td>
<td>Uncertain</td>
</tr>
</tbody>
</table>

Friedman E et al. Br J Cancer. 2015 PMID: 25633036
Case #3 – Is this neoplastic?

- Female in her 70s, AJ ancestry
- Personal and family history of Breast Cancer
  - Mother with breast cancer in her 70s
  - Father with lung cancer in his 70s
- TP53, Exon 8, NM_000546.5:c.839G>C (p.Arg280Thr)
  - Confirmed twice
TP53, Exon 8, NM_000546.5:c.839G>C (p.Arg280Thr)

- VUS, not observed as a germline variant
- Reported as a somatic mutation in multiple types of tumors including breast cancer, bladder cancer, glioma, nasopharyngeal carcinoma and hematopoietic (PMID: 22999923, 1631151, 22187033, 16885334, COSMIC).
- Patient age and family history not consistent with Li Fraumeni
- Patient age and common somatic variant raise suspicion for a hematopoietic neoplasm
Wrapping up
Caveats on Mosaic Results

• It can still be an artifact
  » Many factors can contribute to a bias representation of the alleles
    • PCR/Capture bias and others
  » Does it repeat? Multiple samples? Affected tissue?

• Undiagnosed hematological malignancy
  » Pay attention to age, Fam Hx and other symptoms
  » Reversion of gene change in WBCs can lead to FN

• Could represent undisclosed history of bone marrow transplantation
  » With incomplete engraftment.
Circulating Tumor DNA (ctDNA, liquid biopsy)

- Could a mosaic result represent circulating tumor DNA from a solid tumor?

- Solid tumors release small amounts of mutant DNA in blood
- Detection of ctDNA requires special collection tubes and enrichment of sample for cell free DNA (cfDNA)
- Sensitivity of routine clinical NGS assays for inherited disorders do not achieve required sensitivity to detect ctDNA
Implications for Counseling

• Recurrence risk is a challenge
  » Most of the time the germline status is unknown
  » Germline mosaicism can be considered in most “de novo” cases
    • Suspected in cases with 2 or more affected children
  » More challenging to infer in cancer cases
    • Unknown frequency of mosaicism (tip of the iceberg?)
  » Germline transmission of mutations considered “lethal” from an affected mosaic person should be impossible

• Challenge for genotype–phenotype correlations and prediction of disease manifestations and severity
  » Constitutional and mosaic phenotypes might be different (HRAS-Costello Sx vs. benign keratinocytic epidermal nevi and cancer predisposition)
QUESTIONS?