Mosaic genetic variants in hereditary germline genetic testing: the expected and the unexpected

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Invitae
Learning objectives

1. Define types of mosaic variants

2. Explore possible significance of unexpected mosaic variants in next-generation sequencing assays, including early constitutional mosaicism versus acquired change in a hematopoietic stem cell

3. Understand limitations of germline genetic testing in patients with hematopoietic malignancies; review considerations for clinical interpretation
Mosaicism

Mosaicism is very common: X-inactivation
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, though both populations derive from a single zygote
Chimerism: single organism composed of genetically distinct cells

Mosaics and chimeras

Types of mosaicism

1. **Germline**
   - De novo mutational event in all/some zygotes
   - *Not* in somatic tissues

2. **Somatic** – De novo mutational event in some somatic tissues

3. **Gonosomal** – A combination of germline and somatic mosaicism

Mutations can include point mutations, copy-number variation, chromosomal alterations (rearrangements), whole-chromosome aneuploidy/trisomy, epigenetic modification, etc.
Constitutional mosaicism

Germline  Somatic  Gonosomal

Phenotypic manifestations

**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].
Detection of mosaicism: Sanger (dideoxy) DNA sequencing
Detection of mosaicism: Sanger (dideoxy) DNA sequencing

20% involvement is needed to detect reliability
Detection of mosaicism: Next-generation sequencing, point mutations/small indels
Detection of mosaicism:
Next-generation sequencing, point mutations/small indels

Detection of mosaicism: Next-generation sequencing, point mutations/small indels

- 5–10% involvement is needed to detect reliably and to differentiate from error
- If it is a known mutation, can detect <1/100–1 million, depending on the read depth
Detection of mosaicism: 
Next-generation sequencing, copy number variation

If the NGS pipeline is set to detect germline variants:

- Up to 25% reads or 50% cellular involvement is necessary for detection
- Significantly more or less and it won’t detect
Possible technical sources of mosaicism

1. Polymerase error – Depending on the polymerase can be $10^{-3}$ to $10^{-6}$ errors/nucleotide inclusion

2. Differential affinity of target pull down between wild type and mutant
Germline mosaicism

- Due to post-zygotic de novo mutations affecting either all or a subset of germline cells
  - Male germline cells (as well as epithelial and hematopoietic lineages) are vulnerable to somatic mutations due to repeat divisions
- Highly variable rates of inheritance risk between different autosomal dominant disorders due to
  - Different biological consequences for germ cells (e.g., fetal lethal/decreased fetal viability)
- In patients with apparent de novo copy number variants, germline mosaics are found in 4% of unaffected parents
- In second study, apparent de novo demonstrated that 3.8% of mutations are parental germline mosaic, with 1.3% of mutations being shared between siblings
- Percentage of recurrent mutations between siblings increases to 24% if >1% involvement in peripheral blood in an unaffected parent and to 50% if >6% involvement in peripheral blood
- 4.2% of parents with de novo single nucleotide children who were found to have germline mutations also had same mutation in >1% of blood cells (gonosomal)

(Aside) Review of embryology

Two independent sites generate hematopoietic stem cells:

**Yolk sac (mesodermal)** – The first site of hematopoiesis, generating early myeloid (granulocytes) and erythroid (red cell) elements

**Aortic/gonad/mesonephros region** – The source of self-renewing, multipotent adult hematopoietic stem cells with B- and T-cell-differentiating ability

Somatic mosaicism

- \( \sim 10^{16} \) mitotic cell divisions are required to generate an adult
- Baseline mutation frequency is \( \sim 10^{-8} \) for single nucleotide
- Highest rate of cell proliferation/turnover in hematopoietic and epithelial cells
- 46 nonsense variants, 17 splice site variants, and 52 frame shift indels/person, with likely pathogenic ramifications in adults

Erickson, RP. Mutat Res. 2010 Oct;705(2):96-106.
Somatic mutations in cancer: Adenoma>carcinoma sequence

Schematic of the morphologic and molecular changes in the adenoma-carcinoma sequence

Also present in solid tumors

Timing is everything

Low-level constitutional mosaicism of de novo BRCA1 mutation

- First reported case of BRCA1 constitutional mosaicism
- Ashkenazi Jewish and Bulgarian origin; triple-negative unilateral invasive breast cancer at 43 years
- FHx=

*Sanger sequencing at other lab showed no mutations in BRCAs*

Low-level constitutional mosaicism of de novo BRCA1 mutation

- Hereditary cancer predisposition gene panel ordered (29 genes)
- Pathogenic c.1953dupG (p.Lys652Glufs*21) BRCA1 mutation found and confirmed in 5% of reads
- No other pathogenic mutations found

Low-level constitutional mosaicism of de novo BRCA1 mutation

- Other tissues tested (breast tissue tested by outside commercial lab)

<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>
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<tbody>
<tr>
<td>Blood (draw 1, extraction 1)</td>
<td>164</td>
<td>3307</td>
<td>5.0</td>
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<tr>
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<td>5.2</td>
<td>10.3</td>
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<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>

- Deep sequencing of mother’s blood showed no mutation

- Conclusions
  - Individual constitutional mosaic
  - Mutation likely driving tumor development
  - Repercussions for risk to offspring and counseling regarding contralateral breast cancer and ovarian cancer risk

Clonal hematopoiesis

- Hematopoietic cancer arises from multiple somatic mutations occurring over years
- On average, 1.3 ± 0.2 somatic exonic mutations are acquired per hematopoietic stem cell per decade
- Analysis of inactivation patterns showed that age-related skewing is common in heme stem cells after age 55–65

Clonal hematopoiesis

- Clonal mosaicism (~5–10%) for large chromosomal abnormalities (CNV/LOH) reflecting expansion of clone seen in 1.4-3% elderly (0.5% <50yrs)
- 3–9.5% of clonal mosaic carriers had hematologic malignancy
- 5.5–10x increased risk of developing heme malignancies in unaffected carriers (>2Mb)

Clonal hematopoiesis

- 12,380 participants demonstrated 3111 putative somatic mutations across the genome (one putative somatic mutation for every four participants)
  - Detected allele fraction down to 5%
- Further considered specific mosaic variants seen recurring (>7 patients) in COSMIC database associated with hematolymphoid malignancies

Clonal hematopoiesis (mosaicism x 3) for smaller indel and single nucleotide detected in 10% of individuals older than 65 years and in 1% of individuals younger than 50 years

**Clonal hematopoiesis** – Three or more somatic mutations in a mosaic fashion

**Candidate driver** – A mutation in a heme-related gene seen in at least seven patients in the COSMIC database

Clonal hematopoiesis

Significantly higher hazard ratio for hematologic neoplasm development when clonal hematopoiesis +/- known driver.

1% risk of hematologic cancer/year when clonal hematopoiesis

Clonal hematopoiesis

Significantly higher hazard ratio for hematologic neoplasm development when clonal hematopoiesis +/- known driver.

Clonal hematopoiesis of indeterminate potential (CHIP)

Clonal hematopoiesis of indeterminate potential (CHIP)

- Absence of cytopenia or definitive morphologic evidence of a hematopoietic neoplasm
- Does not meet diagnostic criteria for PNH, MGUS, or MBL
- Presence of a somatic mutation associated with hematological neoplasia at a variant allele frequency of at least 2% (e.g., DNMT3A, TET2, JAK2, SFB3B1, ASKL1, TP53, CBL, GNB1, BCOR, U2AF1, CREBBP, CUX1, SRDF2, MLL2, SETD2, SFDB1, GNAS, PPM1D, BCORL1)

### Genes with reported pathogenic mosaics at Invitae

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>25 (0.09%)</td>
</tr>
<tr>
<td>CHEK2</td>
<td>13 (0.05%)</td>
</tr>
<tr>
<td>ATM</td>
<td>12 (0.05%)</td>
</tr>
<tr>
<td>NF1</td>
<td>10 (0.06%)</td>
</tr>
<tr>
<td>BRCA1</td>
<td>4 (0.016%)</td>
</tr>
<tr>
<td>PTCH1</td>
<td>3 (0.03%)</td>
</tr>
<tr>
<td>MLH1</td>
<td>2 (0.02%)</td>
</tr>
<tr>
<td>FANCI</td>
<td>1</td>
</tr>
<tr>
<td>ALK</td>
<td>1</td>
</tr>
<tr>
<td>APC</td>
<td>1</td>
</tr>
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<td>DSC2</td>
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</tr>
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<td>NBN</td>
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<tr>
<td>RASA1</td>
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<td>RYR2</td>
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<td>SDHA</td>
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<td>SPAST</td>
<td>1</td>
</tr>
<tr>
<td>SPRED1</td>
<td>1</td>
</tr>
</tbody>
</table>

- We’ve seen 83 reports with pathogenic mosaic mutations (0.3% of all patients tested)
- TP53 and ATM seen mutated in CLL (15% and 11%, respectively)
- NF1 is a condition with considerable known mosaicism (~10%)
- Mosaic is ~5-10 to 30%
- All confirmed either by Sanger sequencing or by a second independent NGS
Distribution of age at testing, all reports
Four BRCA1 pathogenic/likely pathogenic mosaics:

1. **Pathogenic** – 5% involvement in PB-Hx breast cancer
2. **Pathogenic** – 12% involvement in PB-Hx breast cancer
3. **Likely Pathogenic** – 10% involvement in PB-Hx colon cancer
4. **Pathogenic** – 28%* involvement in PB-Hx ovarian cancer
## Genes with reported mosaics at Invitae

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of samples</th>
<th>Difference in frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>25</td>
<td>0.09% in Invitae cohort vs. 0.03% in CHIP-normal individuals*</td>
</tr>
<tr>
<td>CHEK2</td>
<td>13</td>
<td>0.05% in Invitae cohort vs. 0% in CHIP-normal individuals*</td>
</tr>
<tr>
<td>ATM</td>
<td>12</td>
<td>0.05% in Invitae cohort vs. 0.008% in CHIP-normal individuals*</td>
</tr>
<tr>
<td>NF1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>BRCA1*</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>PTCH1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

*If seen in >7 patients in COSMIC related to hematolymphoid malignancy
Patient age with mosaicism vs. background testing
Role of TP53 mutations in the origin and evolution of therapy-related hematopoietic malignancies

- TP53 mutations are enriched in therapy-related myeloid neoplasms (t-AML and t-MDS)

- The total number of somatic mutations is equal between t-AML and de novo AML, so chemotherapy does not cause long-term genome-wide DNA damage

- TP53 mutations at t-AML/MDS diagnosis have been identified at low levels (0.003–0.7%) in leukocytes 3–6 years before the development of t-AML/MDS

- Mouse bone marrow chimeras with TP53 mutation had an expanded TP53 mutation pool after exposure to chemotherapy

Role of TP53 mutations in the origin and evolution of therapy-related hematopoietic malignancies

Supports model whereby rare hematopoietic stem cells with age-related TP53 mutations are selected for by chemotherapy/radiation treatment

Patient age with mosaicism vs. background testing

The image shows box plots for different genes (TP53, NF1, CHEK2, ATM) across different age groups (25, 50, 75). The plots compare results between 'Not ATM-CHEK2-TP53' and 'ATM-CHEK2-TP53' conditions.
Role of TP53 mutations in the origin and evolution of therapy-related hematopoietic malignancies

Low-level constitutional mosaicism of de novo TP53 mutation
Suggested clinical follow-up after mosaic result for hereditary cancer predisposition

• Investigate possible constitutional/gonosomal somatic mosaicism in the patient
  – Test skin or other tissue
    – Study shows that buccal swabs/mouthwash can rarely be significantly contaminated with peripheral blood (up to 70% of nucleated cells are WBC)
  – Correlate with genetic results of tumor testing
  – Test offspring to show gonosomal mosaicism (vs in hematopoietic cells)

• Investigate possible acquired somatic mutation in hematopoietic stem cells
  – Review CBC for cytopenia/cytosis, with follow-up studies if appropriate
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

Circulating tumor cells

- Up to three in a million WBC (0.00003%) circulating tumor cells in the blood of cancer patients with active disease

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Total no. of samples</th>
<th>Range of CTCs per mL</th>
<th>Samples with &gt;5 CTCs per ml (%)</th>
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</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>20</td>
<td>0-0-0-0</td>
<td>0-0-0-0</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>55</td>
<td>11-11-11-22</td>
<td>100</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>19</td>
<td>3-8-1-7</td>
<td>100</td>
</tr>
<tr>
<td>Localized prostate cancer</td>
<td>7</td>
<td>0-3-0-4</td>
<td>100</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>15</td>
<td>1-3-2-9</td>
<td>100</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>10</td>
<td>2-0-4-4</td>
<td>100</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>10</td>
<td>0-2-4-3</td>
<td>90</td>
</tr>
</tbody>
</table>

- In studies, up to 0.01% of cellular-free DNA from tumor cells

Germline genetic testing with a background of hematopoietic malignancies (expected mosaics): Considerations for interpretation
Normal blood
Leukemia
Hematopoiesis

STEM CELLS

CLP

Pre-T cell

Pre-B cell

BFU-E CFU-E

Meg-CFC

Mast-CFC

Eo-CFC

GM-CFC

M-CFC

Oc-CFC (?)

COMMITTED PROGENITORS

T-Lymphocyte

B-Lymphocyte

Erythrocyte

Megakaryocyte

Basophil

Eosinophil

Neutrophil

Monocyte/
Macrophage/
Kupffer cell
Langerhans cell
Dendritic cell

MATURE CELLS

/Plasma cell

/Platelets

/Mast cell

/osteoclast

Lymphoid

Myeloid
Acute leukemia

STEM CELLS

COMMITTED PROGENITORS

MATURE CELLS

CLP

Pre-T cell

T-Lymphocyte

B-Lymphocyte /Plasma cell

Erythrocyte

Megakaryocyte /Platelets

Basophil /Mast cell

Eosinophil

Neutrophil

Monocyte/ Macrophage/ Kupffer cell Langerhans cell Dendritic cell

Osteoclast

CMP

Pre-B cell

GM-CFC

M-CFC

Self-renewal

BFU-E

CFU-E

Meg-CFC

Mast-CFC

Eo-CFC

G-CFC

Acute leukemia

Lymphoid

Myeloid
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 neoplasm

STEM CELLS

COMMITTED PROGENITORS

MATURE CELLS

- Pre-T cell → T-Lymphocyte
- Pre-B cell → B-Lymphocyte/Plasma cell
- BFU-E → Erythrocyte
- CFU-E
- Meg-CFC → Megakaryocyte/Platelets
- Mast-CFC → Basophil/Mast cell
- Eo-CFC
- GM-CFC
- Oc-CFC (?) → Monocyte/Macrophage/Kupffer cell/Langerhans cell/Dendritic cell/Osteoclast
- G-CFC
- M-CFC

Lymphoid

Myeloid

Chronic neoplasm
Chronic circulating hematopoietic neoplasms: Lymphoid

1. **B-cell**
   - Chronic lymphocytic leukemia
   - Follicular lymphoma
   - Marginal lymphoma
   - Mantle lymphoma
   - Hairy cell lymphoma
   - Lymphoplasmacytic lymphoma

2. **T-cell**
   - Large granular lymphocytic leukemia
   - Chronic natural-killer cell (NK-cell) leukemia
   - Adult T-cell leukemia/lymphoma
   - Sezary syndrome
   - T-cell prolymphocytic leukemia (usually aggressive; rarely, it can be chronic)
Chronic circulating hematopoietic neoplasms: Myeloid

Neutrophils, eosinophils, basophils, and monocytes in peripheral blood

1. **Myelodysplastic syndrome (MDS)**
   - Cytopenias (low counts)
   - Dysplasia

2. **Myeloproliferative neoplasms (MPN)**
   - Examples include chronic myeloid leukemia, essential thrombocytosis, polycythemia vera, and primary myelofibrosis.
   - Cytoses (high counts—red cell, white cell, and/or platelets)

3. **MDS/MPN neoplasms**
   - Examples include chronic myelomonocytic leukemia, atypical CML, and juvenile myelomonocytic leukemia (JMML)
Limitations of inherited-disease testing in individuals with hematopoietic malignancies

- False positives – acquired mutations
- False negatives
  a) Acquired loss of the chromosomal region containing the germline mutation
     - 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
Limitations of inherited-disease testing in individuals with hematopoietic malignancies (continued)

- False negatives
  - b) Acquired chromosome translocation/insertion/inversion causes misaligning of NGS data

1. Evaluate the likelihood for a false positive or false negative in genes of interest

   a) Determine whether the inherited gene of interest may be affected by hematopoietic neoplasm. (Specific hematopoietic neoplasms have recurrent cytogenetic and molecular changes.)

### Recurrent cytogenetic changes in CLL

<table>
<thead>
<tr>
<th>Frequent Cytogenetic Finding</th>
<th>Percent Involvement</th>
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<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>
Considerations when interpreting inherited-disease genetic testing results in patients with hematopoietic neoplasm

<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>

- Atlas of Genetics and Cytogenetics in Oncology and Hematology ([http://atlasgeneticsoncology.org/index.html](http://atlasgeneticsoncology.org/index.html))
Considerations when interpreting inherited-disease genetic testing results in patients with hematopoietic neoplasm

### Locations of some commonly tested inherited-disease predisposition genes

<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-q23</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>
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<tr>
<td>CDK4</td>
<td>12q14</td>
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<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>
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<td>MET</td>
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<td>4q32</td>
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<td>PMS2</td>
<td>7p22</td>
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<td>PTCH1</td>
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<td>10q23</td>
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<td>RAD51C</td>
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<td>TPS3</td>
<td>17p13</td>
</tr>
<tr>
<td>VHL</td>
<td>3p25</td>
</tr>
</tbody>
</table>
Considerations when interpreting inherited-disease genetic testing results in patients with hematopoietic neoplasm

b) Review available karyotype/FISH testing to determine if the patient’s neoplasm has a gain or loss of chromosomal region/gene or translocation, which may affect inherited-disease testing. **Neoplasms can attain any acquired change, even when they are not recurrent.**
Considerations when interpreting inherited-disease genetic testing results in patients with hematopoietic neoplasm

Can’t definitively rule out change/deletion based on cytogenetics/FISH results.

Cytogenetic testing may not capture all changes that are due to

- a cryptic change for which a FISH probe was not tested
- technical aspects of karyotype assay (metaphase cells analyzed)
- evolution of disease or emergence of a low-level clone since the time of cytogenetic testing
- loss of heterozygosity in a tumor, which masks allele loss

Why “loss of heterozygosity is typical of cancer progression”
Considerations when interpreting inherited-disease genetic testing results in patients with hematopoietic neoplasm

2. Determine the degree of involvement of background neoplasm. (Sometimes, percent involvement can be obtained; other times, it can be difficult to ascertain.)

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

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

   c) For myeloid disorders, the percentage of non-lymphoid white cells (neutrophils, monocytes, eosinophils, basophils) can give the upper limit of degree of involvement. Flow cytometry is not useful.
Considerations when interpreting inherited-disease genetic testing results in patients with hematopoietic neoplasm

d) Ancillary tests may be useful in determining the degree of involvement

   - FISH testing on unselected interphase cells for known acquired change (e.g., T14;18 in follicular lymphoma [50/200 cells])

   - Quantitative molecular tests (e.g., RT-PCR for BCR-ABL in CML)

Karyotyping is not useful because it only detects abnormal metaphases
Considerations when interpreting inherited-disease genetic testing results in patients with hematopoietic neoplasm

3. Determine the degree of sensitivity to detection and the reporting structure of mosaic changes in the lab performing the test.

a) The sensitivity of detection is dependent on
   – the depth of sequencing (number of reads)
   – the lab’s bioinformatic algorithms

b) Reporting is dependent on
   – assay validation
   – the detection threshold/policy set
Interpreting inherited-disease genetic testing results in patients with hematopoietic neoplasm (continued)

4. Correlate with other findings
   a) SNPs in a gene can indicate heterozygosity of allele
      • A BRCA2-benign SNP would indicate that both alleles are detectable
      • If an SNP is present in ~50/50 allele balance, both alleles are likely present in the germline ratio
   b) Family studies can conclude whether the variant present is germline
      • For example, if a variant is found in a family member, can rule out as acquired
   c) Correlate with testing on the tumor or a skin biopsy.
      • For example, a variant present in a tumor in a heterozygous/homozygous (secondary to LOH) fashion suggests germline

Studies show that, rarely, buccal swabs and mouthwash can be significantly contaminated by peripheral blood (up to 70% of nucleated cells are white blood cells)
Illustrative case: hereditary-cancer predisposition germline testing in a patient with a background of chronic lymphocytic leukemia
Chronic lymphocytic leukemia (CLL)

- Neoplastic disorder of B-cells in peripheral blood

- Incidence of 2–6 cases per 100,000 people per year

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

- Patients are diagnosed using a combination of CBC with differential, flow cytometry (morphology can support), and FISH/IPOX testing
Routine 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
Frequent cytogenetic finding | Percent involvement
--- | ---
13q deletion | 55%
Trisomy 12 | 17%
11q deletion | 17%
17p deletion | 7%

BRCA2 is deleted in 80% of patients with a 13q deletion (approximately 45% of patients with CLL)
1. TP53 sequencing
   - Smaller TP53 mutations seen in 8.5–15% of CLL cases
   - TP53 mutations associated with a worse outcome
CLL prognostic testing: a case study

- 64-year-old female with CLL
- Mild anemia only, so no chemotherapy for CLL
- CBC one week prior to NGS blood draw showed 60% lymphocytes
- Flow cytometry performed three weeks prior to NGS blood draw demonstrated 50% of lymphocytes were kappa-light chain-restricted, with an immunophenotype consistent with CLL
  - Approximately 30% of cells were neoplastic CLL cells
- Patient also has a 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
CLL prognostic testing: results

- Pathogenic TP53 point mutation is in a mosaic fashion.
- BRCA2 copy-number variation was normal.
1. Consider the likelihood of a false positive or a false negative
   – False positive = TP53
   – False negative = BRCA2 or other

2. Review the patient’s disease-related testing
   – Karyotype = normal
   – FISH = positive for 13q deletion in 64/200 cells (~30% of cells)
   – BRCA2 is deleted in 80% of patients with a 13q deletion (~45% CLL)

3. Determine degree of involvement
   – CBC = 60% lymphocytes
   – Flow cytometry = 50% of lymphocytes
   – CLL = 30%

4. Determine sensitivity of detection of mosaic findings
   – 5–10% by lab’s report for point mutation/small indels
   – 25% for CNV
CLL prognostic testing: results

1. Pathogenic TP53 point mutation
   a) Mutation present in 14% of alleles, which is consistent with heterozygosity in CLL cells (30% of cells)
   b) Can’t rule out constitutional mosaicism or technical artifact
   c) Would suggest family testing/correlation with a tumor/skin biopsy to explore further
2. BRCA2 copy number variation was normal
   a) Signal was below baseline, but it did not meet the threshold for calling deletion
   b) Copy number variation detection is less sensitive for mosaic detection so can’t rule out BRCA2 del in subset of cells (~15% of alleles)
   c) Take comfort that a lab would detect germline pathogenic point/small indel mutations, even if BRCA2 deleted in CLL cells (41% of alleles would be mutant if mutated in a patient’s germline)
   d) Several benign heterozygous SNPs with a high frequency in the population present in BCRA2 in patient in 50/50 allele ratio
   e) Skin biopsy/fibroblast culture could be performed if clinically necessary
Take-away points

1. Mosaic findings are increasingly prevalent, secondary to increased sensitivity of detection using NGS technology

2. Mosaic findings can be secondary to technical artifact, early constitutional mosaicism, or later-acquired somatic change

3. Clonal hematopoiesis of indeterminate prognosis is a precursor to hematopoietic neoplasm, with 0.5–1% transformation risk/year

4. TP53 mosaic findings may indicate possible precursor to therapy-related myeloid neoplasm in a patient with a history of chemotherapy

5. Patients with circulating hematolymphoid neoplasms can be tested for inherited cancer predispositions using peripheral blood, but results must be carefully interpreted with an understanding of limitations
Thank you!

Questions?