Addressing the challenges of genomic characterization of hematologic malignancies using microarrays

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What is cytogenetics

• The original whole genome analysis
  – Analysis of chromosomes from a tissue of interest to identify large scale genomic alterations
    • G-banded karyotype
  
  – Molecular cytogenetics analyzes smaller regions for imbalances and rearrangements
    • FISH and **genomic microarray**
Indications for an oncology-related chromosome analysis

- Diagnostic chromosome rearrangements
  - CML and t(9;22)

- Prognostic rearrangements

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**Table 6**
Prognostic Implications of Genetic Alterations in Childhood Precursor B Lymphoblastic Leukemia

<table>
<thead>
<tr>
<th>Cytogenetic finding</th>
<th>Genetic alteration</th>
<th>Frequency</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(9;22)(q34;q11.2)</td>
<td>BCR/ABL</td>
<td>3–4%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>t(4;11)(q21;q23)</td>
<td>AF4/MLL</td>
<td>2–3%</td>
<td>Unfavorable</td>
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<tr>
<td>t(1;19)(q23;p13.3)</td>
<td>PBX1 (PBX1/E2A)</td>
<td>6% (25% of pre B-all)</td>
<td>Unfavorable&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>t(12;21)(p13;q22)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>TEL/AML1</td>
<td>16–29%</td>
<td>Favorable</td>
</tr>
<tr>
<td>Hyperdiploid &gt;50</td>
<td></td>
<td>20–25%</td>
<td>Favorable</td>
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<tr>
<td>Hypodiploidy</td>
<td></td>
<td>5%</td>
<td>Unfavorable</td>
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</table>
Indications for a oncology-related chromosome analysis

- Monitoring of secondary changes that may signal disease progression

<table>
<thead>
<tr>
<th>Additional change</th>
<th>Frequency (%)</th>
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</thead>
<tbody>
<tr>
<td>+Ph</td>
<td>15</td>
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<tr>
<td>i(17q)</td>
<td>12</td>
</tr>
<tr>
<td>+8</td>
<td>11</td>
</tr>
<tr>
<td>+Ph, +8</td>
<td>8</td>
</tr>
<tr>
<td>+8,i(17q)</td>
<td>7</td>
</tr>
<tr>
<td>+Ph,+8,+19</td>
<td>5</td>
</tr>
<tr>
<td>+Ph,+19</td>
<td>4</td>
</tr>
<tr>
<td>+8,+19</td>
<td>2</td>
</tr>
<tr>
<td>+Ph,+8,i(17q)</td>
<td>2</td>
</tr>
<tr>
<td>+19</td>
<td>1</td>
</tr>
<tr>
<td>i(17q),+Ph</td>
<td>1</td>
</tr>
<tr>
<td>+8,i(17q),+19</td>
<td>1</td>
</tr>
<tr>
<td>+Ph,+8,i(17q),+19</td>
<td>1</td>
</tr>
<tr>
<td>i(17q),+19</td>
<td>&gt;1</td>
</tr>
<tr>
<td>i(17q),+19,+Ph</td>
<td>&gt;1</td>
</tr>
</tbody>
</table>

\(^a\text{der(22)t(9;22)(q34.1;q11.2).}\)
Monitoring effectiveness of therapy

- Disappearance of previously detected chromosome rearrangement - good
- Appearance of new chromosome rearrangements – not good

Secondary hematologic malignancies

Most common tissue studied: bone marrow/peripheral blood for leukemias/lymphomas; tissue biopsy for solid tumors
Standard Karyotyping

G-banding (Giemsa) chromosomes in metaphase

Benefits:
  - Viewing entire genome
  - Can visualize individual cells and individual chromosomes

Limits:
  - Limit of resolution around 5-10 Mb (depending on region of genome and length of chromosomes)
  - Need an actively growing source of cells
Microarray should not be considered “the test” in all cancer samples

- Balanced translocations may need to be ruled out based on indication
  - CML and BCR/ABL1 fusion
  - APL and PML/RARA fusion

- As mosaicism detection not reliable under 20-30% (depending on size and direction), not recommended for minimal residual disease detection

- Not currently designed to detect specific nucleotide mutations

- Consider utility in cases in which CN and aLOH is informative (CLL, MDS, ALL, AML, multiple myeloma, renal cell carcinoma) and supplement as needed with other tests
Overcome the preferential growth of nonmalignant cells

Normal karyotype in all metaphase cells from a patient with acute lymphoblastic leukemia
Cytoscan HD shows +21, +X and a small deletion of IKZF1
Deletion of *IKZF1* and Prognosis in Acute Lymphoblastic Leukemia

**CONCLUSIONS**

Genetic alteration of *IKZF1* is associated with a very poor outcome in B-cell–progenitor ALL.

*IKZF1* deletions predict relapse in uniformly treated pediatric precursor B-ALL

RP Kuiper, E Waanders, VHJ van der Velden, SV van Reijmersdal, R Venkatachalam, B Scheijen, E Sonneveld, JJM van Dongen, AJP Veerman, FN van Leeuwen, A Geurts van Kessel, and PM Hoogerbrugge

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Detection and characterization of recurrent translocations play an important role in diagnosis and treatment of hematological disorders.

Chromosomal microarray analysis (CMA) is a powerful tool to detect copy number changes in hematological disorders. One of the limitations of CMA platforms currently in use is that truly balanced chromosome rearrangements cannot be detected.

However, some chromosomal rearrangements have cryptic losses or gains at the breakpoints which may be detected by microarray. In addition, recurrent balanced translocations followed by gain or loss of one of the derivative chromosomes can be detected by CMA, and recurrent interstitial deletions resulting in gene fusions may also detected by microarray.
Case 1: Focus on the subtle copy number and or allele pattern transition points

NOT just mosaic trisomy 8
Really have two der(8)t(8;22) with MYC/IGK fusion plus one normal 8 and one normal 22 and one der 22 – cytogenetically can be hard to distinguish from trisomy 8
ETV6 deletion – an indicator for translocation?

7 ALL cases showed ETV6 deletion on array, 5 were unbalanced translocations involving ETV6, 4 partnering with RUNX1
Native *ETV6* deletions accompanied by *ETV6-RUNX1* rearrangements are associated with a favourable prognosis in childhood acute lymphoblastic leukaemia: a candidate for prognostic marker

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Example: Better breakpoint characterization

ALL with apparently balanced rearrangements

46,XY,der(10)t(10;12)(q22.1;p13),der(12)t(10;12)(q22.1;p13)t(12;13)(q24.3;q14),der(13)t(12;13)(q24.3;q14)[5]/46,XY[15]
With Cytoscan HD, multiple deletions were detected around the breakpoints.

Can evaluate genes in intervals for known roles as either fusion or deletion products in ALL.
CASE 2: 15 yo Male  B-ALL

46,XY,der(13;der14)(q10;q10),t(14;17)(q32;q21)[2]/46,XY[18]
CASE 2

Low level gain of 14q and 17q. The gene distal to the breakpoint on 17 is \textbf{IGF2BP1}.

nuc ish (5’ \textit{IGH}@x2, 3’ \textit{IGH}@x3)(5’ \textit{IGH}@ con 3’ \textit{IGH}@x1)[30/200]
CASE 2

47,XY,+10,t(14;17)(q32;q21)[15].

pathogenetically essential aberration

high IGF2BP1 expression is a characteristic feature of ETV6/RUNX1-positive leukemic blasts
Example: Clinically relevant copy number alterations may all be cytogenetically cryptic

46,XY[25].nuc ish (IGH@x2)(5'IGH@ sep 3'IGH@x1)[139/200], (MYC,CDKN2A,MLL,TCF3)x2[200]
9q34(ABL1x2),22q11(BCRx2) [200]
Identification of potential constitutional abnormalities

• ATRX – somatic mutations
  – From www.GeneReviews.org

  **Alpha-Thalassemia X-Linked Intellectual Disability Syndrome;** Roger E Stevenson, MD, FACMG Clinical Genetics and Cytogenetics; Greenwood Genetic Center

  **Disease characteristics.** Alpha-thalassemia X-linked intellectual disability (ATRX) syndrome is characterized by distinctive craniofacial features, genital anomalies, severe developmental delays, hypotonia, intellectual disability, and mild-to-moderate anemia secondary to alpha-thalassemia.

  Acquired mutation also possible:
• STS gene deletion in male predicted to result in X-linked ichthyosis (XLI), which results from steroid sulfatase deficiency (see www.omim.org #308100).

• Recommend genetic counseling and constitutional study
No chromosome analysis, ALL FISH Panel only

ABNORMAL FISH RESULTS
  nuc ish 8q24(MYC)x3[112/200]
  9q34(ABL1)x3[124/200]

NORMAL FISH RESULTS
  nuc ish 11q23(MLL)x2
  14q32(IGH@x2)
  19p13.3(TCF3)x2
  22q11.2(BCRx2)
Whole genome array results suggestive of diagnosis of MDS/MPD.

- Chr 3: complex loss/gain
- Chr 5: deletion of 5q
- Chr 7: complex 7q loss/gain
- Chr 8: trisomy
- Chr 20: complex loss/gain
- Chr 21: trisomy
- Chr 9: trisomy plus LOH of 9p

Homozygous JAK2 (9p24.1) mutation associated with aLOH 9p observed in ~37% of MPDs.

Klampf T et al., Blood, 2011.
Common patterns of CNV and aLOH in MPN

Klampfl T et al., Blood, 2011.
Common patterns of aLOH in MDS and AML

Figure 4. Genomic distribution of acquired CN-LOH in MDS/secondary AML and primary AML. CN-LOH is nonrandomly distributed across the genome in both MDS/secondary AML (blue lines) and primary AML (red lines), with some chromosomes and chromosomal regions being more frequently affected.
Multiple studies identifying mutated tumor suppressors or oncogenes selected by aLOH

Table 1. Mutated genes in regions of UPD

<table>
<thead>
<tr>
<th>Mutated gene</th>
<th>Region of UPD</th>
<th>Described</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPL</td>
<td>UPD1p</td>
<td>MPN, RARSt</td>
<td>52</td>
</tr>
<tr>
<td>NRAS</td>
<td>UPD1p</td>
<td>JMML, CMML</td>
<td>49</td>
</tr>
<tr>
<td>TET2</td>
<td>UPD4q</td>
<td>sAML, MPN, MDS/MPD,</td>
<td>53, 54</td>
</tr>
<tr>
<td>JAK2</td>
<td>UPD9p</td>
<td>PV, ET, IMF, RARSt</td>
<td>55-57</td>
</tr>
<tr>
<td>CDKN2B</td>
<td>UPD9p</td>
<td>ALL</td>
<td>4</td>
</tr>
<tr>
<td>WT1</td>
<td>UPD11p</td>
<td>AML</td>
<td>6</td>
</tr>
<tr>
<td>c-CBL</td>
<td>UPD11q</td>
<td>CMML, MDS/MPN, JMML</td>
<td>49, 50, 58-60</td>
</tr>
<tr>
<td>FLT3/ITD</td>
<td>UPD13</td>
<td>AML</td>
<td>6, 49</td>
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<tr>
<td>TP53</td>
<td>UPD17p</td>
<td>MDS, sAML</td>
<td>61</td>
</tr>
<tr>
<td>NF1</td>
<td>UPD17q</td>
<td>JMML</td>
<td>62</td>
</tr>
<tr>
<td>CEBPa</td>
<td>UPD19q</td>
<td>AML</td>
<td>6, 63</td>
</tr>
<tr>
<td>RUNX1</td>
<td>UPD21q</td>
<td>AML</td>
<td>6</td>
</tr>
</tbody>
</table>

RARSt indicates refractory anemia with ring sideroblasts in transformation; JMML, juvenile myelomonocytic leukemia; sAML, secondary acute myeloid leukemia; PV, polycythemia vera; ET, essential thrombocytopenia; and IMF, idiopathic myelofibrosis.
Acute lymphoblastic leukemia: 45YM
No chromosome analysis

nuc ish 8q24(MYCx4)[47/200]
  9q34(ABL1x2),22q11.2(BCRx3)[28/200]
  11q23(MLLx4)[34/200]
  14q32(IGH@x4)[33/200]
  19p13.3(TCF3x4)[23/200]

FISH results were reported as consistent with hyperdiploidy – which is a good prognosis

Allele pattern can distinguish true origin of copy number
– critical in ALL and MM
Chromosomes with 4 copies by FISH, had allele pattern consistent with both homologs gained.

Allele plot at middle shows allelic balance so one homolog not gained over other.

Chromosomes with 2 copies by FISH showed loss of homozygosity – consistent with one homolog lost, other homolog gained.
Conclusion:

1. Consistent with *Severe Hypodiploid ALL* with doubling of the hypodiploidy clone.

2. The pattern of allele difference is necessary for correct interpretation.

Hypodiploidy is a very poor prognosis – the allele pattern resulted in a change in the prognosis and treatment.
Multiple studies indicate poor yield of MDS FISH subsequent to normal cytogenetics.

Table 1: Results of Metaphase Cytogenetics and FISH in 433 Cases*

<table>
<thead>
<tr>
<th>Metaphase Cytogenetics</th>
<th>FISH Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (20 metaphase cells)</td>
<td>6/222 (2.7%)</td>
</tr>
<tr>
<td>Suboptimal growth (&lt;20 metaphase cells)</td>
<td>6/43 (14%)</td>
</tr>
<tr>
<td>No growth</td>
<td>10/54 (19%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>72/114 (63.2%)</td>
</tr>
</tbody>
</table>

FISH, fluorescence in situ hybridization.
* Data are given as number/total (percentage).

The utility of fluorescence in situ hybridization analysis in diagnosing myelodysplastic syndromes is limited to cases with karyotype failure.

Hui Jiang, Yongquan Xue, Qinrong Wang, Jinlan Pan, Yafang Wu, Jun Zhang, Shuxiao Bai, Qian Wang, Guangsheng He, Aining Sun, Depei Wu, Suning Chen*
Much better diagnostic yield from SNP-based array for MDS, MPN, sAML
Chronic Lymphocytic Lymphoma: The perfect target

- Genetic lesions of known importance involve losses or gains
- Copy number variation stratifies cases
- Fresh samples readily available
- Tumor burden is typically determined by Flow cytometry
Correlation of FISH and Microarray – Overall 98%

<table>
<thead>
<tr>
<th></th>
<th>FISH</th>
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<tr>
<td>Overall</td>
<td>correlation</td>
<td>25/26 (96%)</td>
<td>24/25 (96%)</td>
<td>24/25(96%)</td>
<td>25/25 (100%)</td>
<td>25/25 (100%)</td>
</tr>
</tbody>
</table>

Microarray replaced FISH for clinical study.
Coming soon – ACMG Guidelines

Section E15 of the American College of Medical Genetics and Genomics technical standards and guidelines: Microarray Analysis for Chromosome Abnormalities in Neoplastic Disorders

Cooley, Linda D. MD, MBA1; Lebo, Matthew PhD2; Li, Marilyn M PhD3; Slovak, Marilyn L PhD4; Wolff, Daynna JPhD5; A Working Group of the American College of Medical Genetics and Genomics (ACMG) Laboratory Quality Assurance Committee

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