Reporting cytogenetics

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Reporting cytogenetics

• What is it?
• Terminology
• Clinical value
• What details are important
Diagnostic Tools

• Microscope  What do the cells (blasts) look like? How do they stain?

• Flow Cytometry  fluorescent antibody measure of molecules and density on cells

• Cytogenetics  Chromosome number, structure and changes

• Molecular testing (PCR)  DNA or RNA changes that indicate the tumor cells
**Immunocytochemistry**

M5
Strongly positive for the nonspecific esterase inhibited by Fluoride.

M5
Chloroacetate esterase stains neutrophils blue, nonspecific esterase stains monocytes red-brown.
Diagnosis - Immunocytochemistry

MPO and PAS (red) in normal BM

M7
Factor VIII related protein identifies the blasts as being of megakaryocyte lineage.

MPO in M2 (orange)

PAS
Reporting cytogenetics

• How are they tested?

• What is FISH?

• What’s the difference?

• What do they mean?
Reporting cytogenetics

• How are they tested?
  Structural and numerical changes in chromosomes—while cells are dividing

• What is FISH? *Fluorescent in situ hybridization*
  Specific markers on defined chromosome sites

• What’s the difference?
  Dividing (metaphase) vs. non-dividing (interphase)

• What do they mean?
Specimen requirements

• Cytogenetics
  – SODIUM heparin (green top)
  – Core biopsy acceptable (in saline, RPMI or other media)
  – FFPE tissue acceptable for FISH UNLESS it has been decalcified

• G-bandning
  – Requires dividing cells to be able to examine chromosomes during metaphase

• FISH
  – Cells need not be dividing
Analysis

• 20 metaphase cells are analyzed by G-bandning

• FISH frequently used to confirm presence of a specific gene rearrangement and provides means for monitoring response to therapy
  – FISH can examine hundreds of cells
Clone

• 2 or more cells with gain of a specific chromosome

• 2 or more cells with the same structural chromosomal abnormality

  -- OR --

• 3 or more cells with loss of a specific chromosome
FLUORESCENCE IN SITU HYBRIDIZATION (FISH)
TECHNICAL STEPS

Labeled Probe

Target

Denature

Hybridize

Detect

Hybridize

Visualize
Advantages of FISH

• Targets relatively stable DNA within the cell
• Quantitates genetic changes cell-by-cell
• Simultaneous assessment of multiple genetic targets in an intact cell
• Easy to perform
• Short time-to-result
• Equipment generally available in most laboratories
Types of FISH probes

• Centromere enumeration probes
  – To monitor number of a specific chromosome in a cell
Karyotype: Chromosomes in pairs; numbered by size
47,XY,+8

47 chromosomes, male
Gain (extra) chromosome 8
CEP 8
CEP6 (hybridization control)
Types of FISH probes

• Locus specific probes
  – To rule out deletions, gains or rearrangements of specific loci
Dual fusion probes

- Used to confirm presence of a translocation
- Fusion signal on each partner (derivative) chromosome
- Highly specific (very low false positive rate)
46, XX, t(9;22) (q34;q11.2)

46 chromosomes, female

Translocation between chromosome 9 and 22 parts of each long arm exchanged

balanced translocation: no net gain or loss of material
46,XX,t(9;22)(q34;q11.2)
t(9;22)

Bcr Abl
t(9;22)

Bcr Abl
Breakapart probes

- Used to confirm rearrangements of genes
- 3’ portion of gene or region in one color, 5’ in another

If rearranged, colors are separated
inv(16)(p13q22)
46,XY,inv(16)(p13.1; q22)

46 chromosomes, male
Inversion of piece between short and long arm of chromosome 16
**FISH**

- AML M3, t(15;17)
- AML M1, trisomy 8

**AML M4Eo-Inv 16**

**Normal Cell**

**Chromosome 15**

**Chromosome 17**

**Abnormal Cell with t(15;17)**

**Chromosome 15**

**Fusion Product t(15;17)**

**Chromosome 17**

**Inverted Chromosome 16**

**Normal Chromosome 16**

**Chromosome 8**

**Chromosome 7 (control)**

**Abnormal Cell**

**Chromosome 7 (control)**

**Chromosome 8**
46,XY,t(9;11)(p22;q23)

46 chromosomes, male
Translocation between 9 and 11
short arm 9 and long arm 11
46,XX,t(6;9)(p23;q34)

46 chromosomes, female
Translocation between 6 and 9
short arm 6 and long arm 9
Clone 1: 44,XY,der(5)add(5)(p15.1)add(5)(q31),-6,del(7)(q21q34),
inv(10)(p11.2q21),del(11)(q21q23),-13,der(16)t(16;17)(p11.2;q11.2),-17,
der(18)t(18;21)(p11.2;q11.2),add(19)(q13.3),-20,-21,der(22)t(11;22)(q13;p11.2),
+mar1,+mar2,+mar4
46, XY, der(5)add(5)(p15.1) add(5)(q31), -6, del(7)(q21q34), inv(10)(p11.2q21), del11(q21q23), -13, der(16)t16;17)(p11.2;q11.2), -17, der(18)t(18;21)(p11.2;q11.2), add(19)(q13.3), -20, -21, der(22) t(11;22)(q13;p11.2), +mar1, +mar2, +mar4
46,XY,der(5)add(5)(p15.1)
add(5)(q31),-6,del(7)(q21q34),
inv(10)(p11.2q21),del11(q21q23)
-13,der(16)t16;17)(p11.2;q11.2),
-17,der(18)t(18;21)(p11.2;q11.2)
add(19)(q13.3),-20,-21,der(22)
t(11;22)(q13;p11.2),
+mar1,+mar2,+mar4

complex
Clone 1: 44,XY,der(5)add(5)(p15.1)add(5)(q31),-6,del(7)(q21q34),
        inv(10)(p11.2q21),del(11)(q21q23),-13,der(16)t(16;17)(p11.2;q11.2),-17,
        der(18)t(18;21)(p11.2;q11.2),add(19)(q13.3),-20,-21,der(22)t(11;22)(q13;p11.2),
        +mar1,+mar2,+mar4
36 Were cytogenetics tested (conventional or FISH)?

☐ yes ☐ no ☐ Unknown

37 Date sample collected: _______ - ______ - ______

38 Results of tests

☐ Abnormalities identified

☐ No evaluable metaphases

☐ No abnormalities
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<tr>
<th>Chromosome</th>
<th>Abnormality</th>
<th>Specified?</th>
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<tr>
<td>39</td>
<td>-5</td>
<td>yes</td>
</tr>
<tr>
<td>40</td>
<td>-7</td>
<td>no</td>
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<tr>
<td>41</td>
<td>-17</td>
<td>yes</td>
</tr>
<tr>
<td>42</td>
<td>-18</td>
<td>yes</td>
</tr>
<tr>
<td>43</td>
<td>-X</td>
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<tr>
<td>44</td>
<td>-Y</td>
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Trisomy

45  +4

☐  yes  ☐  no

46  +8

☐  yes  ☐  no

47  +11

☐  yes  ☐  no

48  +13

☐  yes  ☐  no

49  +14

☐  yes  ☐  no

50  +21

☐  yes  ☐  no

51  +22

☐  yes  ☐  no
Translocation

52  t(3;3)
   □ yes □ no

53  t(6;9)
   □ yes □ no

54  t(8;21)
   □ yes □ no

55  t(9;11)
   □ yes □ no

56  t(9;22)
   □ yes □ no

57  t(15;17) and variants
   □ yes □ no

58  t(16;16)
   □ yes □ no
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<tr>
<th>Deletion</th>
<th>59</th>
<th>del(3q) / 3q-</th>
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<th>60</th>
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<th>61</th>
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<th>62</th>
<th>del(9q) / 9q-</th>
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<tr>
<th>63</th>
<th>del(11q) / 11q-</th>
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<tr>
<th>64</th>
<th>del(16q) / 16q-</th>
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<tr>
<th>67</th>
<th>del(21q) / 21q-</th>
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</table>
**Inversion**

68 inv(3)

- Yes
- No

69 inv(16)

- Yes
- No

**Other**

70 (11q23) any abnormality

- Yes
- No

71 12p any abnormality

- Yes
- No

72 Complex - ≥ 3 distinct abnormalities

- Yes
- No
73 Other abnormality

☐ yes ☐ no

74 Specify other abnormality: ______________

75 Was documentation submitted to the CIBMTR?
   (e.g. cytogenetic or FISH report)

☐ yes ☐ no
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<th>Question</th>
<th>Yes</th>
<th>No</th>
<th>Unknown</th>
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<tr>
<td>76 Were tests for molecular markers performed (e.g. PCR)?</td>
<td>☐ yes</td>
<td>☐ no</td>
<td>☐ Unknown</td>
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<tr>
<td>77 Date sample collected:</td>
<td>__________ - __________</td>
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<td></td>
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<tr>
<td>78 CEBPA</td>
<td>☐ Positive</td>
<td>☐ Negative</td>
<td>☐ Not Done</td>
</tr>
<tr>
<td>79 FLT3 – D835 point mutation</td>
<td>☐ Positive</td>
<td>☐ Negative</td>
<td>☐ Not Done</td>
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<tr>
<td>80 FLT3 – ITD mutation</td>
<td>☐ Positive</td>
<td>☐ Negative</td>
<td>☐ Not Done</td>
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<td>81 IDH1</td>
<td>☐ Positive</td>
<td>☐ Negative</td>
<td>☐ Not Done</td>
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<td>82 IDH2</td>
<td>☐ Positive</td>
<td>☐ Negative</td>
<td>☐ Not Done</td>
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<td>83 KIT</td>
<td>☐ Positive</td>
<td>☐ Negative</td>
<td>☐ Not Done</td>
</tr>
<tr>
<td>84 NPM1</td>
<td>☐ Positive</td>
<td>☐ Negative</td>
<td>☐ Not Done</td>
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Prognostic Groups by Cytogenetics
CIBMTR SWOG Modified by ELN 2012

- **Good prognosis:** t(15;17), inv (16), del(16q), t(16;16)
- t(8;21) without del(9q) or complex

- **Intermediate:** Normal Karyotype, -Y, +8, +6, del (12p), t(9;11), 11q23 MLL rearranged, any abnormality neither good or poor risk

- **Unfavorable (Poor) Prognosis:** abnormal 3, -5, -7, abn11, t(6;9), t(9;22), complex karyotype (≥3 abnormalities)
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• Good prognosis: t(15;17), inv (16), del(16q), t(16;16) t(8;21) without del(9q) or complex

• Intermediate : Normal Karyotype, -Y, +8, +6, del (12p), t(9;11), 11q23 MLL rearranged, any abnormality neither good or poor risk

• Unfavorable (Poor) Prognosis: abnormal 3, -5, -7, abn11, t(6;9), t(9;22), complex karyotype (≥3 abnormalities)
<table>
<thead>
<tr>
<th>Genetic Group</th>
<th>Description</th>
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<tbody>
<tr>
<td>Favorable</td>
<td>t(8;21)(q22;q22); RUNX1-RUNX1T1</td>
</tr>
<tr>
<td></td>
<td>inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11</td>
</tr>
<tr>
<td></td>
<td>Mutated NPM1 without FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td>Intermediate-I</td>
<td>Mutated CEBPA (normal karyotype)</td>
</tr>
<tr>
<td>Intermediate-II</td>
<td>Mutated NPM1 and FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td></td>
<td>Wild-type NPM1 and FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td></td>
<td>Wild-type NPM1 without FLT3-ITD (normal karyotype)</td>
</tr>
<tr>
<td>Adverse</td>
<td>abnormallyes not classified as favorable or adverse</td>
</tr>
<tr>
<td></td>
<td>inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1</td>
</tr>
<tr>
<td></td>
<td>t(6;9)(p23;q34); DEK-NUP214</td>
</tr>
<tr>
<td></td>
<td>t(v;11)(v;q23); MLL rearranged</td>
</tr>
<tr>
<td></td>
<td>−5 or del(5q), −7, abnl(17p)</td>
</tr>
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<td></td>
<td>Complex karyotype*</td>
</tr>
</tbody>
</table>

*Mrózek K, JCO 2012
MRC-AML 10 trial- OS by Cytogenetics

Wheatley K et al, Brit J Haem 1999
Outcome of patients with primary acute myeloid leukemia classified into the four European LeukemiaNet genetic groups according to the European LeukemiaNet recommendations.

Mrózek K et al. JCO 2012;30:4515-4523