Reporting cytogenetics

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University of Minnesota
Reporting cytogenetics

• What is it?
• Terminology
• Clinical value
• What details are important
“The bloodwork came back kinda yucky.”
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

MPO in M2 (orange)

M7
Factor VIII related protein identifies the blasts as being of megakaryocyte lineage.
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-banding
- 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
What’s a karyotype

- Add tissue sample
- Add chemical to stimulate mitosis
- Incubate for 2-3 days
- Add chemical to stop mitosis in metaphase
- Transfer cells to tube and centrifuge to concentrate in layers
- Culture in a growth medium
- Cut out chromosome pictures and arrange into karyotype
- Identify and photograph chromosomes
- Add stain to enhance chromosomes
- Transfer to tube containing fixative
Dividing Cells
Chromosomes are spread out
Photographs of stained chromosomes lined up by size (number)
Size and banding pattern identifies each chromosome
FLUORESCENCE IN SITU HYBRIDIZATION (FISH)
TECHNICAL STEPS

Labeled Probe

Target

Denature
Hybridize
Detect
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,XY,+8
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
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 Chromosome 16

Inverted Chromosome 16
46,XY,t(9;11)(p22;q23)
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
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,\text{der}(5)\text{add}(5)(p15.1)
\text{add}(5)(q31),-6,\text{del}(7)(q21q34),
\text{inv}(10)(p11.2q21),\text{del}11(q21q23)
-13,\text{der}(16)t16;17)(p11.2;q11.2),
-17,\text{der}(18)t(18;21)(p11.2;q11.2)
\text{add}(19)(q13.3),-20,-21,\text{der}(22)
t(11;22)(q13;p11.2),
+\text{mar}1,+\text{mar}2,+\text{mar}4
Clone 1: 44, XY, der(5)t(5;14)(p15.1;q21.1), 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
Form 2010 R3.0: Acute Myelogenous Leukemia (AML) Pre-HCT Data

Center: 
CRID: 

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
Specify cytogenetic abnormalities identified at diagnosis: Monosomy

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<tr>
<td>39</td>
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<td>44</td>
<td>-Y</td>
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</table>
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
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
Deletion

59 del(3q) / 3q–
   □ yes □ no

60 del(5q) / 5q–
   □ yes □ no

61 del(7q) / 7q–
   □ yes □ no

62 del(9q) / 9q–
   □ yes □ no

63 del(11q) / 11q–
   □ yes □ no

64 del(16q) / 16q–
   □ yes □ no

65 del(17q) / 17q–
   □ yes □ no

66 del(20q) / 20q–
   □ yes □ no

67 del(21q) / 21q–
   □ yes □ no
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
<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
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<tbody>
<tr>
<td>76</td>
<td>Yes, No, Unknown</td>
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<tr>
<td>77</td>
<td>Date sample collected: <strong>-</strong>-__</td>
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<tr>
<td>78</td>
<td>CEBPA: Positive, Negative, Not Done</td>
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<tr>
<td>79</td>
<td>FLT3 – D835 point mutation: Positive, Negative, Not Done</td>
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<td>80</td>
<td>FLT3 – ITD mutation: Positive, Negative, Not Done</td>
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<td>81</td>
<td>IDH1: Positive, Negative, Not Done</td>
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<td>82</td>
<td>IDH2: Positive, Negative, Not Done</td>
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<td>83</td>
<td>KIT: Positive, Negative, Not Done</td>
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<tr>
<td>84</td>
<td>NPM1: Positive, Negative, 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)
<table>
<thead>
<tr>
<th>Genetic Group</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Favorable</strong></td>
<td>t(8;21)(q22;q22); RUNX1-RUNX1T1</td>
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<tr>
<td></td>
<td>inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11</td>
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<td>Mutated NPM1 without FLT3-ITD (normal karyotype)</td>
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<td></td>
<td>Mutated CEBPA (normal karyotype)</td>
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<tr>
<td><strong>Intermediate-I</strong></td>
<td>Mutated NPM1 and FLT3-ITD (normal karyotype)</td>
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<td>Wild-type NPM1 and FLT3-ITD (normal karyotype)</td>
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<tr>
<td></td>
<td>Wild-type NPM1 without FLT3-ITD (normal karyotype)</td>
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<tr>
<td><strong>Intermediate-II</strong></td>
<td>t(9;11)(p22;q23); MLLT3-MLL</td>
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<tr>
<td></td>
<td>abnormalities not classified as favorable or adverse</td>
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<tr>
<td><strong>Adverse</strong></td>
<td>inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1</td>
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<tr>
<td></td>
<td>t(6;9)(p23;q34); DEK-NUP214</td>
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<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>
<tr>
<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
Outcomes 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

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"This is a teaching hospital."