Reporting cytogenetics
Can it make sense?

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Reporting cytogenetics

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

• 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
Diagnosis - Immunocytochemistry

MPO and PAS (red) in normal

MPO in M2 (orange)

M7
Factor VIII related protein identifies blasts of megakaryocyte lineage.
Immunocytochemistry

M5
Strongly positive for the nonspecific esterase Inhibited by Fluoride.

M5
Chloroacetate esterase stains neutrophils blue, nonspecific esterase stains monocytes red-brown
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?
  Molecular probes to find chromosome changes
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 can be used to monitor 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

1. Culture in a growth medium
2. Add tissue sample
3. Add chemical to stimulate mitosis
4. Incubate for 2-3 days
5. Add chemical to stop mitosis in metaphase
6. Transfer cells to tube and centrifuge to concentrate in layers
7. Put cells onto microscope slide
8. Add stain to enhance chromosomes
9. Cut out chromosome pictures and arrange into karyotype
10. Identify and photograph chromosomes
11. 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

AGTAGGGTC

TCATCAACAG

AGTAGGTGTC

TCATCAACAG

Denature
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 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, 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

*Philadelphia chromosome*
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
ISCN: 46,XY,inv(16)(p13.1q22)
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
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 complex
46,XY,der(5)add(5)(p15.1)
add(5)(q31),-6,del(7)(q21q34),
inv(10)(p11.2q21),del11(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
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
Was the disease status assessed by cytogenetic testing (karyotyping or FISH)?

Were cytogenetic abnormalities identified via FISH?
- Yes - Go to question 26
- No - Go to question 29

Date sample collected: ___ ___ ___ ___ — ___ ___ — ___ ___

Specify abnormalities
- -5
- -7
- -17
- -18
- -X
- -Y
- +4
- +8
- +11
- +13
- +14
- +21
- +22

monosomy

translocation
- t(3;3)
- t(6;9)
- t(8;21)
- t(9;11)
- t(9;22)
- t(15;17) and variants
- t(16;16)
Were cytogenetic abnormalities identified via karyotyping?

Then

Same questions
Molecular testing

Were tests for molecular markers performed (e.g. PCR, NGS)?

☐ Yes – *Go to question 47*
☐ No – *Go to question 57*
CEBPA

☐ Positive – *Go to question 48*
☐ Negative - *Go to question 49*
☐ Not done - *Go to question 49*

Specify CEBPA mutation
☐ Biallelic (homozygous)
☐ Monoallelic (heterozygous)
☐ Unknown

Repeat format for:

FLT3 – D835 point mutation
FLT3 – ITD mutation
IDH1
IDH2
KIT
NPM1
Pre-TED forms

Specify the AML classification:

**AML with recurrent genetic abnormalities**

- AML with t(9;11) (p22.3;q23.3); MLLT3-KMT2A (5)
- AML with t(6;9) (p23;q34.1); DEK-NUP214 (6)
- AML with inv(3) (q21.3;q26.2) or t(3;3) (q21.3;q26.2); GATA2, MECOM (7)
- AML (megakaryoblastic) with t(1;22) (p13.3;q13.3); RBM15-MKL1 (8)
  - AML with t(8;21); (q22; q22.1); RUNX1-RUNX1T1 (281)
- AML with inv(16)(p13.1;1q22) or t(16;16)(p13.1; q22); CBFB-MYH11 (282)
- APL with PML-RARA (283)
  - AML with BCR-ABL1 (provisional entity) (3)
  - AML with mutated NPM1 (4)
  - AML with biallelic mutations of CEBPA (297)
  - AML with mutated RUNX1 (provisional entity) (298)
  - AML with 11q23 (MLL) abnormalities (i.e., t(4;11), t(6;11), t(9;11), t(11;19)) (284)
Pre-TED forms

AML with recurrent genetic abnormalities
- AML with myelodysplasia – related changes (285)
  - Therapy related AML (t-AML) (9)

AML, not otherwise specified
- AML, not otherwise specified (280)
  - AML, minimally differentiated (286)
- AML without maturation (287)
- AML with maturation (288)
- Acute myelomonocytic leukemia (289)
- Acute monoblastic / acute monocytic leukemia (290)
- Acute erythroid leukemia (erythroid / myeloid and pure erythroleukemia) (291)
- Acute megakaryoblastic leukemia (292)
- Acute basophilic leukemia (293)
  - Acute panmyelosis with myelofibrosis (294)

- Myeloid Sarcoma (295)
Prognostic Groups by Cytogenetics
CIBMTR SWOG Modified by ELN

• 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>Risk category*</th>
<th>Genetic abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>t(8;21)(q22;q22.1); \textit{RUNX1-RUNX1T1}</td>
</tr>
<tr>
<td></td>
<td>inv(16)(p13.1q22) or t(16;16)(p13.1;q22); \textit{CBFB-MYH11}</td>
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<tr>
<td></td>
<td>Mutated \textit{NPM1} without \textit{FLT3-ITD} or with \textit{FLT3-ITD}^\text{low}†</td>
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<td>Biallelic mutated \textit{CEBPA}</td>
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<tr>
<td>Intermediate</td>
<td>Mutated \textit{NPM1} and \textit{FLT3-ITD}^\text{high}†</td>
</tr>
<tr>
<td></td>
<td>Wild-type \textit{NPM1} without \textit{FLT3-ITD} or with \textit{FLT3-ITD}^\text{low}† (without adverse-risk genetic lesions)</td>
</tr>
<tr>
<td></td>
<td>t(9;11)(p21.3;q23.3); \textit{MLLT3-KMT2A}‡</td>
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<tr>
<td></td>
<td>Cytogenetic abnormalities not classified as favorable or adverse</td>
</tr>
<tr>
<td>Adverse</td>
<td>t(6;9)(p23;q34.1); \textit{DEK-NUP214}</td>
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<tr>
<td></td>
<td>t(v;11q23.3); \textit{KMT2A} rearranged</td>
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<tr>
<td></td>
<td>t(9;22)(q34.1;q11.2); \textit{BCR-ABL1}</td>
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<td></td>
<td>inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); \textit{GATA2,MECOM(EVI1)}</td>
</tr>
<tr>
<td></td>
<td>−5 or del(5q); −7; −17/abn(17p)</td>
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<td></td>
<td>Complex karyotype,§ monosomal karyotypell</td>
</tr>
<tr>
<td></td>
<td>Wild-type \textit{NPM1} and \textit{FLT3-ITD}^\text{high}†</td>
</tr>
<tr>
<td></td>
<td>Mutated \textit{RUNX1}$\dagger$</td>
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<tr>
<td></td>
<td>Mutated \textit{ASXL1}$\dagger$</td>
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<tr>
<td></td>
<td>Mutated \textit{TP53}$#$</td>
</tr>
</tbody>
</table>
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
“This is a teaching hospital.”
ELN 2017: AML risks—functional & structural

3 D glasses available on request