Acute Myeloid Leukaemia (AML)

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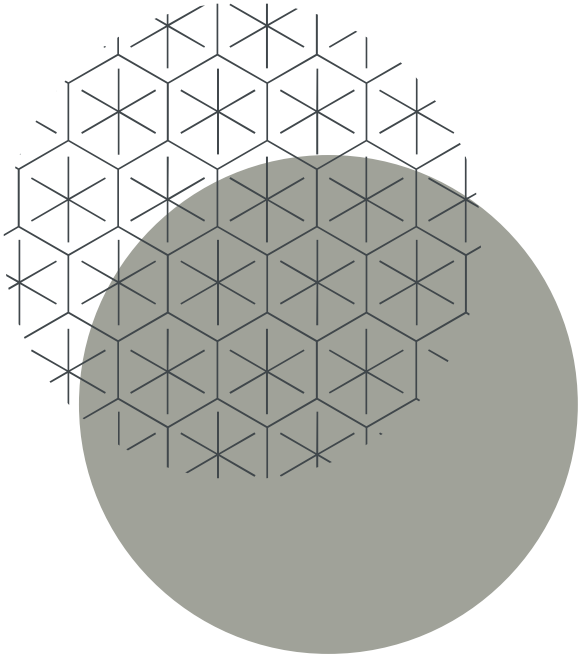
Table of Contents

Abbreviations

BM	Bone marrow
DNA	Deoxyribonucleic acid
FISH	Fluorescence in situ hybridization
MDS	Myeloproliferative syndrome
PB	Peripheral blood
RBC	Red blood cell
WHO	World Health Organization



01

What is AML?

Acute myeloid leukaemia (AML) is caused by a DNA mutation in the stem cells in bone marrow that produce red blood cells, platelets and infection-fighting white blood cells.

The mutation causes the stem cells to produce many more white blood cells than are needed.

The white blood cells produced are still immature, so they do not have the infection-fighting properties of fully developed white blood cells.

As the number of immature cells increases, the amount of healthy red blood cells and platelets decrease, and this causes many of the complications such as fatigue, infections and bleeding (Gorczyca, 2008, p 184; National Health Service, 2019).

Gorczyca, W. (2008). *Cytogenetics, FISH and molecular testing in hematologic malignancies*. CRC Press, 184.
National Health Service. (2019, February 28). *Causes acute myeloid leukaemia.* Retrieved from https://www.nhs.uk/conditions/acute-myeloid-leukaemia/causes/

Risk Factors of AML

- Radiation exposure

Being exposed to a significant level of radiation can increase the chances of developing AML, although this usually requires exposure at very high levels.

- Benzene and smoking

Benzene is found in petrol, and it's also used in the rubber industry. It is also found in cigarette smoke, which could explain why people who smoke have an increased risk of developing AML.

- Previous cancer treatment (radiotherapy & chemotherapy)

- Genetic disorder (Down syndrome & Fanconi's anemia)

National Health Service United Kingdom. (2019, February 28). *Causes acute myeloid leukaemia.* Retrieved from https://www.nhs.uk/conditions/acute-myeloid-leukaemia/causes/

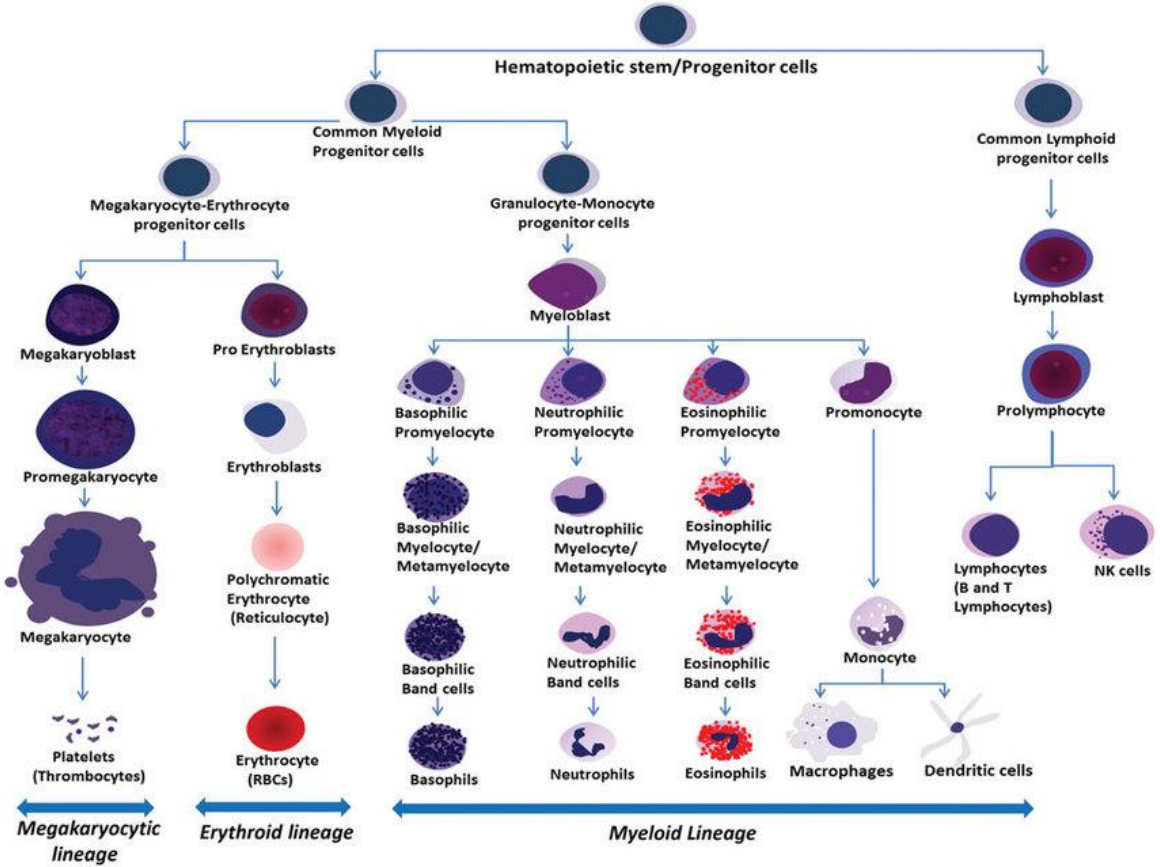


Diagram 1 Hierarchy of hematopoietic cells during normal differentiation of bone marrow-derived hematopoietic stem/progenitor cells into lineage-specific blood cells.

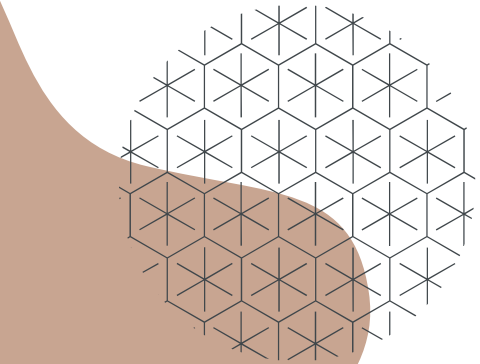
Note. This diagram was produced by Mahalingaiah et al. in 2018, summarizing the differentiation of bone-marrow derived stem/progenitor cells into hematopoietic lineages.

02

FAB Classification of AML

French-American-British (FAB) Classification of AML

FAB subtype	Name
M0	Undifferentiated acute myeloblastic leukaemia
M1	Acute myeloblastic leukaemia with minimal maturation
M2	Acute myeloblastic leukaemia with maturation
M3	Acute promyelocytic leukaemia (APL)
M4	Acute myelomonocytic leukemia
M4 eos	Acute myelomonocytic leukaemia with eosinophilia
M5	Acute monocytic leukaemia
M6	Acute erythroid leukaemia
M7	Acute megakaryoblastic leukaemia



FAB classification based on:

- The type of cell the leukaemia develops from
- How mature the cells are

- ✓ Subtypes M0 through M5 all start in immature forms of white blood cells.
- ✓ M6 AML starts in very immature forms of red blood cells.
- ✓ M7 AML starts in immature forms of cells that make platelets.

American Cancer Society. (2018, August 21). *Acute myeloid leukemia (AML) subtypes and prognostic factors.* Retrieved from https://www.cancer.org/cancer/acute-myeloid-leukemia/detection-diagnosis-staging/how-classified.html#references

03

WHO Classification of AML

WHO Classification of AML

AML with t(8;21)(q22;q22); *RUNX1::RUNX1T1*

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB::MYH11*

Acute promyelocytic leukaemia with t(15;17)(q22;q12); *PML::RARA*

AML with t(9;11)(p22;q23); *MLLT3::MLL*

AML with t(6;9)(p23;q34); *DEK::NUP214*

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1::EVI1*

AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15::MKL1*

AML with mutated *NPM1*

AML with mutated *CEBPA*

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 109

3.1 AML with t(8;21)(q22;q22); *RUNX1::RUNX1T1*

Definition

- Is an AML generally showing maturation in the neutrophil lineage.

Epidemiology

- Found in ~5% of cases of AML.
- Occurs predominantly in younger patients.

Genetics

- The t(8;21)(q22;q22) involves the *RUNX1* gene, which encodes core-binding factor subunit alpha and the *RUNX1T1 (ETO)* gene.
- The *RUNX1::RUNX1T1* fusion transcript is consistently detected in patients with t(8;21)(q22;q22) AML.
- Known as AML M2.

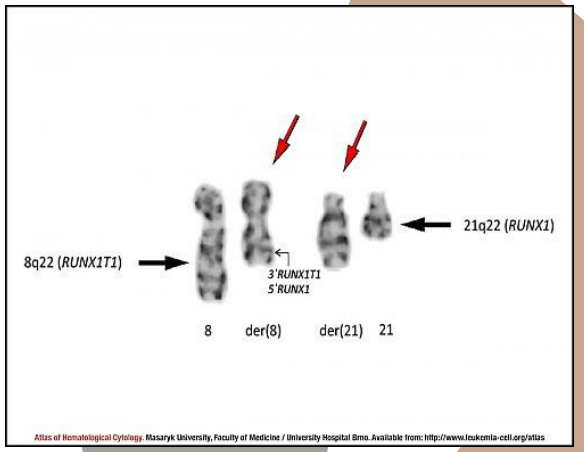


Figure 1 G-banded partial karyotype of translocation t(8;21)(q22;q22)
This partial karyotype demonstrates translocation t(8;21)(q22;q22). Red arrows indicate derivative chromosomes 8 and 21. Breakpoint sites are indicated by black arrows. The t(8;21)(q22;q22) results in a juxtaposition of the *RUNX1T1* gene (formerly known as *ETO*) located on 8q22 and the *RUNX1* gene (formerly known as *AML1*) located on 21q22. This juxtaposition generates the fusion gene *RUNX1-RUNX1T1* located on the der(8) chromosome. Image credit: https://www.leukemia-cell.org/

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 110

3.2 AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB::MYH11*

Definition

- Is an AML that usually shows monocytic and granulocytic differentiation and characteristically abnormal eosinophil component in BM.

Epidemiology

- Can be found in all age groups but are found predominantly in younger patients.

Genetics

- The inv(16)(p13.1q22) found in vast majority and the less common t(16;16)(p13.1;q22).
- Both result in fusion of the CBFB gene at 16p22 to the MYH11 gene at 16p13.1.
- Known as AML M4eo.

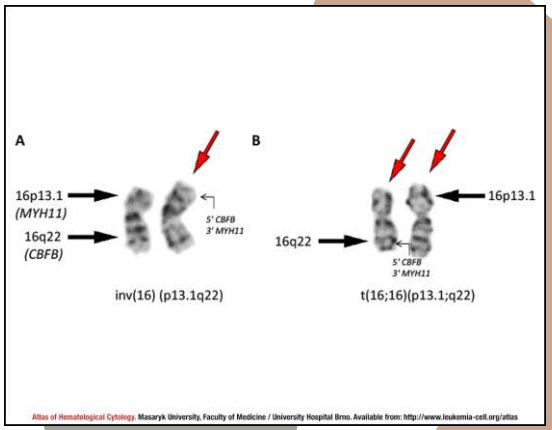


Figure 2 G-banded partial karyotype of inversion inv(16)(p13.1q22) and translocation t(16;16)(p13.1;q22)

A: The inversion results from breakage and rejoining of bands 16p13.1 and 16q22 on the same chromosome 16. The derivative chromosome 16 is indicated by a red arrow.

B: The translocation t(16;16)(p13.1;q22) results from a breakage at bands 16p13.1 and 16q22 on different chromosomes 16 (the breakpoint sites are indicated by black arrows).

Image credit: https://www.leukemia-cell.org/

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 111.

3.3 Acute promyelocytic leukaemia with t(15;17)(q22;q12); *PML::RARA*

Definition

- Is an AML in which abnormal promyelocytes predominate.

Epidemiology

- Comprises 5-8% of AML.
- Can occur at any age but patients are predominantly adults in mid-life.

Genetics

- Fusion of retinoic acid receptor alpha (RARA) gene on 17q12 with a nuclear regulatory factor gene on 15q22 gives rise to a *PML::RARA* fusion gene product.
- Known as AML M3.

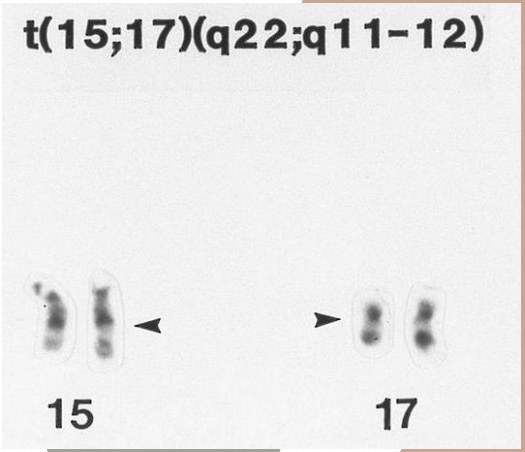


Figure 3 G-banded partial karyotype of t(15;17)(q22;q12)

G-banded partial karyotype demonstrating the translocation t(15;17)(q22;q12). Red arrows indicate derivative chromosomes 15 and 17. Breakpoint sites are indicated by black arrows on abnormal chromosomes. Image credit: https://www.pathologyoutlines.com/

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 112.

3.4 AML with t(9;11)(p22;q23); *MLLT3::MLL*

Definition

- Is an AML that is usually associated with monocytic features.

Epidemiology

- Can occur at any age, but is more common in children.

Genetics

- The *MLL* protein is a histone methyltransferase that assembles in protein complexes that regulate gene transcription via chromatin remodeling.
- The t(9;11)(p22;q23) involving *MLLT3* (9p22) is the most common *MLL* translocation in AML.

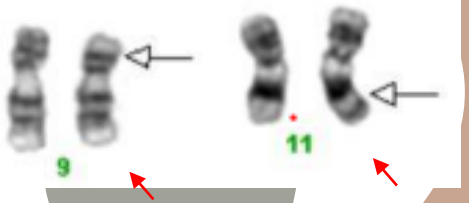


Figure 4 G-banded partial karyotype of t(9;11)(p22;q23)

G-banded partial karyotype demonstrating the translocation t(9;11)(p22;q23). Red arrows indicate derivative chromosomes 9 and 11. Breakpoint sites are indicated by black arrows on normal chromosome homologues

Image credit: American Society of Hematology

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 114.

3.5 AML with t(6;9)(p23;q34); *DEK::NUP214*

Definition

- Is an AML with or without monocytic features that is often associated with basophilia and multilineage dysplasia.

Epidemiology

- Occurs in both children and adults with a median age of 13 years (childhood) and 35 years (adults).

Genetics

- Results in a fusion of *DEK* on chromosome 6 with *NUP214* on chromosome 9.
- The resulting nucleoporin fusion protein acts as an abberant transcription factor and altering nuclear transport by binding to soluble transport factors.

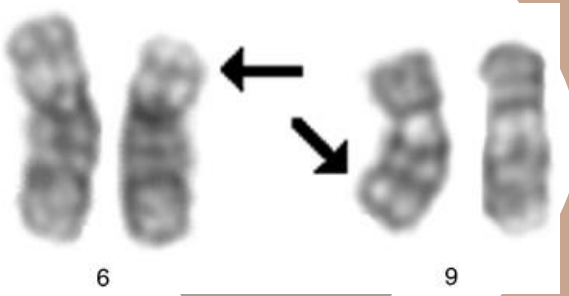


Figure 5 G-banded partial karyotype of t(6;9)(p23;q34)
G-banded partial karyotype demonstrating the translocation t(6;9)(p23;q34). Arrows indicate the locations of the breakpoints on the abnormal chromosomes 6 and 9
Image credit: https://www.nature.com/

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 115.

3.6 AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1::EVI1*

Definition

- Is an AML that may present *de novo* or arise from a prior MDS.
- It is often associated with normal or elevated PB platelet counts and has increased atypical BM megakaryocytes.

Epidemiology

- Common in adults with no sex predilection.

Genetics

- The abnormalities involve the oncogene *EVI1* at 3q26.2, or its longer form *MDS1::EVI1*, and *RPN1* at 3q21.
- *RPN1* may act as an enhancer of *EVI1* expression, resulting in increased cell proliferation, and impaired cell differentiation.

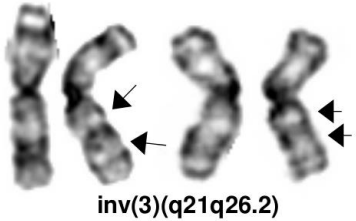


Figure 6 G-banded partial karyotype of inv(3)(q21q26.2)
G-banded partial karyotype demonstrating the inversion inv(3)(q21q26.2)
Arrows indicate the locations of the breakpoints on the abnormal chromosome 3
Image credit: https://ccga.io/

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 116.

3.7 AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15::MKL1*

Definition

- Is an AML generally showing maturation in the megakaryocyte lineage.

Epidemiology

- An uncommon abnormality in AML, representing <1% of all cases.
- Commonly occurs in infants without Down syndrome, with a female predominance.

Genetics

- This translocation results in the fusion of RNA-binding motif protein-15 (*RBM15*) and megakaryocyte leukaemia-1 (*MKL1*).

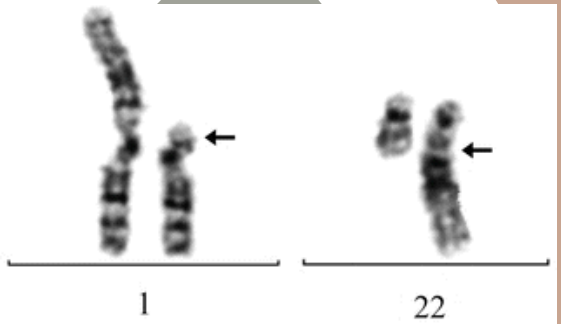


Figure 7 G-banded partial karyotype of t(1;22)(p13;q13)
G-banded partial karyotype demonstrating the translocation t(1;22)(p13;q13). Arrows indicate the locations of the breakpoints on the abnormal chromosomes 1 and 22
Image credit: https://link.springer.com/

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 117.

3.8 AML with mutated *NPM1*

Definition

- Carries mutations that usually involve exon 12 of the *NPM1* gene.
- Aberrant cytoplasmic expression of nucleophosmin is a surrogate marker of this gene mutation.
- Typically presents *de novo* in older adults with a normal karyotype.

Epidemiology

- One of the most common recurring genetic lesions in AML.
- Prevalence increases with age.
- Appears to show a female predominance.

Genetics

- Usually associated with a normal karyotype.
- 5-15% of AML with *NPM1* mutation show chromosomal aberrations such as +8 and del(9q).

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 121.

3.9 AML with mutated *CEBPA*

Definition

- Usually meets the criteria for AML with maturation or without maturation, but some cases may show myelomonocytic or monoblastic features.
- This leukaemia usually present *de novo*.

Epidemiology

- Occurs 6-15% of *de novo* AML and 15-18% of AML with normal karyotype.

Genetics

- 70% of AML with *CEBPA* mutation have a normal karyotype.

Campo, E., Harris, N. L., Jaffe, E. S., Pileri, S. A., Stein, H., Thiele, J., & Vardiman, J. W. (2008). *WHO classification of tumours of haematopoietic and lymphoid tissues* (Vol. 2, p. 439). S. H. Swerdlow (Ed.). Lyon, France: International agency for research on cancer. 122.

04

Prognosis of AML

Table 1 Most common chromosomal abnormalities in AML

Cytogenetic abnormality	*Genes*	*Prognosis*
t(1;3)(p36;q21)	*MEL1::ribophorin*	Poor
t(1;7)(q10;q10)		Poor
t(1;11)(p32;q23)	*AF1p::MLL*	Poor
t(1;11)(p21;q23)	A*F1q::MLL*	Poor
t(1;22)(p13;q13)	*OTT::MAL*	Poor
inv(3)(q21;q26), t(3;3)(q21;q26)	*EVI1::ribophorin*	Poor
t(3;5)(q25.1;q35)	*MLF1::NPM*	Intermediate to poor
t(3;21)(q26;q22)	*EVI1* or *MDS1::RUNX1*	Poor
+4		Poor
del(5q)		Very poor

Gorczyca, W. (2008). *Cytogenetics, FISH and molecular testing in hematologic malignancies*. CRC Press. 184

Table 1 Most common chromosomal abnormalities in AML

Cytogenetic abnormality	*Genes*	*Prognosis*
t(5;17)(q35;q12)	*NPM::RARA*	Poor
t(6;9)(p23;q34)	*DEK::CAN*	Poor
t(6;11)(q27;q23)	*AF6::MLL*	Poor
t(7;11)(p15;p15)	*HOXA9::NuP98*	Intermediate
-7/del(7q)		Very poor
+8		Intermediate to poor
t(8;16)(q11;q13)	*MOZ::CBP*	Poor
t(8;21)(q22;q22)	*ETO::RUNX1*	Good
t(9;11)(p21-22;q23)	*MLLT3::MLL*	Intermediate
t(9;22)(q34;q11)	*BCR::ABL1*	Poor

Gorczyca, W. (2008). *Cytogenetics, FISH and molecular testing in hematologic malignancies*. CRC Press. 184.

Table 1 Most common chromosomal abnormalities in AML

Cytogenetic abnormality	*Genes*	*Prognosis*
t(10;11)(p12;q23)	*AF10::MLL*	Poor
+11	*MLL*	Poor
t(11;16)(q23;p13)	*MLL::CBP*	Poor
t(11;17)(q23;q25)	*MLL::AF17*	Poor
t(11;17)(q23;q21)	*PLZF::RARA*	Intermediate
t(11;17)(q13;q21)	*NUMA::RARA*	Good
t(11;19)(q23;p13)	*MLL::ENL*	Poor
i(12)(p10)		Poor
t(12;22)(p13;q11)	*TEL::MN1*	Poor
+13		Poor
Monosomal		Poor

Gorczyca, W. (2008). *Cytogenetics, FISH and molecular testing in hematologic malignancies*. CRC Press. 184.

Table 1 Most common chromosomal abnormalities in AML

Cytogenetic abnormality	*Genes*	*Prognosis*
t(15;17)(q22;q11)	*PML::RARA*	Good
inv(16)(p13q22), t(16;16)(p13;q22), del(16)(q22)	*MYH11::CBFB*	Good
t(16;22)(p11;q22)	*FUS::ERG*	Poor
i(17)(q10)		Poor
t(17;17)(q11;q21)	*STAT5b::RARA*	
del(20q)		Poor
+21		Intermediate
+22		Intermediate
-Y		Intermediate
Complex (>3)		Very poor

Gorczyca, W. (2008). *Cytogenetics, FISH and molecular testing in hematologic malignancies*. CRC Press. 184.

05

Limitations of Karyotyping

Might not be able to detect:

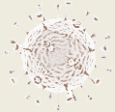
- Subtle or submocroscopic aberrations
- Low level mosaicism
- Submicroscopic genetic changes (e.g. gene mutations)

Requires fresh viable cells (Sampson & McGuire, 2014).

Cell culture is typically required (1–10 days) (Bridge, 2008).

May encounter complex karyotypes with suboptimal morphology (Bridge, 2008).

Has limited resolution, at the level of 5-10 megabases (Vermeech et al., 2007).

Chances of bacterial contamination 

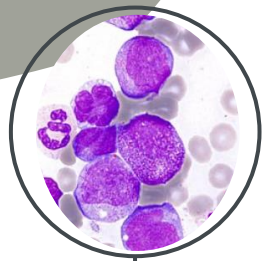
Bridge, J. A. (2008). Advantages and limitations of cytogenetic, molecular cytogenetic, and molecular diagnostic testing in mesenchymal neoplasms. *Journal of Orthopaedic Science*, *13*(3), 273-282. doi: 10.1007/s00776-007-1215-1

Sampson, B., & McGuire, A. (2014). Genetics and the molecular autopsy. *Pathobiology of Human Disease*, 3459–3467. https://doi.org/10.1016/b978-0-12-386456-7.06707-1

Vermeesch, J.R., Fiegler, H., De Leeuw, N., Szuhai, K., Schoumans, J., Ciccone, R., Speleman, F., Rauch, A., Clayton-Smith, J., Van Ravenswaaij, C. & Sanlaville, D. (2007). Guidelines for molecular karyotyping in constitutional genetic diagnosis. *European Journal of Human Genetics*, *15*(11), 1105-1114. DOI: 10.1038/sj.ejhg.5201896

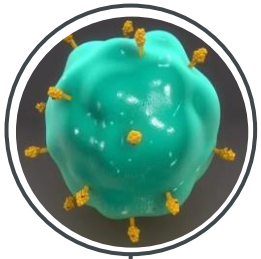
06

Other Tests Available



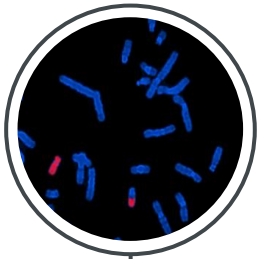
Cytochemistry

Cells are exposed to chemical stains (dyes) that react with only some types of leukemia cells.



Immunophenotyping

To classify leukemia cells according to the substances (antigens) on their surfaces through flow cytometry and/or immunohistochemistry.



FISH

To look for changes in specific genes or parts of chromosomes by using special fluorescent dyes.

Molecular Biology (PCR) and Next generation sequencing (NGS)

To find some gene and chromosome changes too small to be seen under a microscope.

American Cancer Society. (2018, August 21). *Tests for acute myeloid leukemia (AML).* Retrieved from https://www.cancer.org/cancer/acute-myeloid-leukemia/detection-diagnosis-staging/how-diagnosed.html

References

American Cancer Society. (2018, August 21). *Acute myeloid leukemia (AML) subtypes and prognostic factors.* Retrieved from https://www.cancer.org/cancer/acute-myeloid-leukemia/detection-diagnosis-staging/how-classified.html#references

American Cancer Society. (2018, August 21). *Tests for acute myeloid leukemia (AML).* Retrieved from https://www.cancer.org/cancer/acute-myeloid-leukemia/detection-diagnosis-staging/how-diagnosed.html

Bridge, J. A. (2008). Advantages and limitations of cytogenetic, molecular cytogenetic, and molecular diagnostic testing in mesenchymal neoplasms. *Journal of Orthopaedic Science*, *13*(3), 273-282. doi: 10.1007/s00776-007-1215-1

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Thank you