# INTRODUCTION

Leukemia is a disease resulting from the neoplastic proliferation of myeloid or lymphoid cells. It results from a mutation in a single stem cell, the progeny of which forms a clone of leukemic cells (blast cells). It is characterized by proliferation of leukemic cells and their infiltration into normal tissues. Increased cell proliferation has metabolic consequences and infiltrating cells also disturb tissue function. Anemia, neutropenia and thrombocytopenia are important consequences of infiltration of the bone marrow (BM), which, in turn, can lead to infection and hemorrhage.[1] The leukemic blasts circulate into the peripheral blood (PB) and may infiltrate other parenchymatous organs as liver, spleen and lymph nodes (LNs).[2]

Often, there is a series of genetic alterations rather than a single event. Genetic events contributing to malignant transformation include inappropriate expression of oncogenes and loss of function of tumor suppressor genes (TSGs). The cell in which the leukemic transformation occurs may be a lymphoid precursor, a myeloid precursor or a pluripotent stem cell capable of differentiating into both myeloid and lymphoid cells. Myeloid leukemias can arise in a lineage-restricted cell or in a multipotent stem cell capable of differentiating into cells of erythroid, granulocytic, monocytic and megakaryocytic lineages.[1]

Leukemias are broadly divided on basis of disease progression and life expectancy into:[3]

(i) Acute leukemias: a rapidly progressive disease characterized by a defect in maturation, leading to an imbalance between proliferation and maturation; since cells of the leukemic clone continue to proliferate without maturing to end cells and dying. There is continued expansion of the leukemic clone and immature cells predominate (usually blast forms). If untreated, acute leukemias lead to death in weeks or months.
(ii) Chronic leukemias: a slowly progressive disease characterized by an expanded pool of proliferating cells that retain their capacity to differentiate to end cells and thus consists mainly of more mature cells. If untreated, chronic leukemias lead to death in months or years.

Acute leukemias are further divided into acute lymphoblastic leukemias (ALL), acute myeloid leukemias (AML) -also designated acute non-lymphoblastic leukemias- and mixed phenotype acute leukemias (MPAL), the latter showing both lymphoid and myeloid differentiation.[1]

## Acute Myeloid Leukemia

Acute myeloid leukemia is a heterogeneous clonal neoplasm characterized by accumulated genetic aberrations, which result in enhanced proliferation, block in differentiation and increased survival of the leukemic blast cells and variable response to therapy and is the most common malignant myeloid disorder in adults.[4, 5]

It is characterized by an increase in the number of myeloid cells in the BM and an arrest in their maturation, frequently resulting in hematopoietic insufficiency (granulocytopenia, thrombocytopenia, or anemia), with or without leukocytosis.[6]

Acute myeloid leukemia is the most common type of leukemia in adults, yet continues to have the lowest survival rate of all leukemias. Although rates have improved remarkably in the younger age group, the prognosis in older patients continues to be very poor.[7]

## Epidemiology

### Incidence

Acute myeloid leukaemia has an incidence of 2-3 per 100, 000 per year in children, rising to 15 per 100, 000 in older adults. It can occur at all ages but has its peak incidence in the seventh decade. The fact that most cases occur in older patients has important implications for treatment strategies; in that biological variation associated with chemoresistance and comorbidity (which limits treatment options) increases with age.[8]

Although the incidence of acute leukemias acc\ounts for <3% of all cancers, these diseases constitute the leading cause of death due to cancer in children and adults aged <39 years. Acute myeloid leukaemia accounts for approximately 25% of all leukemias in adults in the western world, and, therefore, is the most frequent form of leukemia. Worldwide, the incidence of AML is highest in the U.S., Australia, and Western Europe. The age-adjusted incidence rate of AML in the U.S. between the years 1975–2003 was approximately 3.4 per 100,000 persons.[7]

In Egypt, according to the national cancer institute (NCI) registry, in all ages, both genders, leukemias ranked the fourth together with urinary bladder (UB) cancer (7.2%). In female, for all ages, it constituted 6.5% and coming the second after breast cancer. In males, it is the fourth after liver, UB and lymphomas forming 8% of all cancers for all ages.

The frequency of leukemias between years 2002-2010 in pediatrics was 2718 out of 4262 patients diagnosed with hematological malignancies (63.8%), in adults it was 3699 out of 9371 patients diagnosed with hematological malignancies (39.5%). Regarding AML, there were 2054 patients; 1097 males and 957 females.[9]

### Age

Both the nature of AML and the health of the patient change with age. Older patients are more likely to have more comorbidities and have a poorer performance status than younger patients.[10]

Acute Myeloid Leukemia comprises only 15% to 20% of cases in patients aged <15 years. The peak incidence rate occurs in the first year of life and then decreases steadily up to the age of 4 years, at which point it remains relatively constant throughout the years of childhood and early adulthood. It is therefore primarily a disease of later adulthood. Patients newly diagnosed with AML have a median age of 65 years. AML is rarely diagnosed before the age of 40 years; thereafter, the incidence increases progressively with age.[7]

The prognosis of patients with AML is age-dependent, with chemoresistance being observed more frequently with increasing age. This is supposed to be based on differences in the biology of AML in younger and older patients. Hence, 2 different mechanisms of genetic defects can be distinguished; balanced chromosomal translocations lead to leukemia-specific fusion-transcripts without gain and loss of chromosomal material, whereas unbalanced chromosome abnormalities lead to multiple gains and losses of genetic material. The balanced translocations such as t(15;17), t(8;21), and inv(16)/t(16;16) are reported to be more frequent in younger patients, whereas in elderly patients

unbalanced abnormalities mostly involving complete or partial losses of chromosomes 5 and 7 and, in particular, complex aberrations are detected more often.[11]

In Egypt, between years 2002-2010, age of presentation differ between pediatrics and adults patients being 7 years for pediatrics male and female, and 42 year for adult males and 43 years for adult females.[9]

**Sex and Ethinity**

The incidence of AML varies with gender and race. In the Surveillance, Epidemiology, and End Result (SEER) database for children ages 1 to 4 years, there was an incidence rate of 0.8 per 100,000 persons for boys and for girls. In the first few years of life, the incidence of AML in whites is 3-fold higher than in blacks; however, blacks have slightly higher rates of AML among children age > 3 years. Acute Myeloid Leukemia in adults has a slight male predominance in most countries. In 2000–2003, the age-adjusted incidence rate of AML in the U.S. was 3.7 per 100,000 for both sexes, 4.6 per 100,000 for males, and 3.0 per 100,000 for females. The incidence rate of U.S. males is substantially higher than the incidence rates reported for males in all other countries. In the U.S. in 2000, AML was more common in blacks than in whites. However, during 2000 to 2003, the incidence of AML for blacks (3.2 per 100,000 persons) was lower than for whites (3.8 per 100,000 persons).[7]

**Mortality**

Untreated AML is a uniformly fatal disease. Although it is possible to support patients for a certain period (median survival, 11–20 weeks), they ultimately die of the leading complications associated with BM failure (i.e., infection and hemorrhage). The mortality associated with AML varies with age, gender, and race. Mortality rates in the U.S. appear to increase with age because the age-adjusted mortality rate shows its peak at 17.6 per 100,000 persons in people aged 80 to 84 years. The mortality rate for males is higher than that for females, with the U.S. age-adjusted mortality rate is 3.5 per 100,000 for males and 2.2 per 100,000 for females (2000–2003). Acute Myeloid Leukemia mortality is greater in whites than in blacks. The U.S. age-adjusted mortality rate was 2.7 per 100,000 for whites and 2.2 per 100,000 for blacks in the year 2000.[7]

**Etiology**

Although multiple risk factors have been linked to the development of AML (Table 1), including age, gender, previous chemotherapy, other hematologic disorders, genetic abnormalities, cigarette smoking, and exposures to radiation and benzene, these known risk factors account for only a small number of observed cases.[12]

**Table (1): Selected risk factors associated with AML.**[7]

| | |
|---|---|
| **1- Genetic disorders** | Down syndrome<br>Klinefelter syndrome<br>Patau syndrome<br>Ataxia telangiectasia<br>Shwachman syndrome<br>Kostmann syndrome<br>Neurofibromatosis<br>Fanconi anemia<br>Li-Fraumeni syndrome |
| **2- Physical and chemical exposures** | Benzene<br>Drugs such as pipobroman<br>Pesticides<br>Cigarette smoking<br>Embalming fluids<br>Herbicides |
| **3- Radiation exposure** | Nontherapeutic, therapeutic<br>Radiation |
| **4- Chemotherapy** | Alkylating agents<br>Topoisomerase-II inhibitors<br>Anthracyclines<br>Taxanes |

## Pathophysiology

AML is a malignant clonal disorder of immature cells in the haemopoietic hierarchial system. Leukaemic transformation is assumed to occur in many cases at, or near, the level of the haemopoietic stem cell before it has embarked on any lineage commitment. Some cases may originate at a slightly later stage in cells that are committed to lineage differentiation. These cells have abnormal function characterized by failure to progress through the expected differentiation programme and/or to die by the process of apoptosis. Associated with this may be retention of the stem cell characteristic of self - renewal. This leads to the accumulation of a clone of cells, which dominates BM activity and leads to marrow failure.[8]

## Clinical Presentations

The clinical signs and symptoms of AML are diverse and nonspecific, but they are usually directly attributable to the leukemic infiltration of the BM, with resultant cytopenias. Typically, patients present with signs and symptoms of fatigue, hemorrhage, or infections and fever due to decreases in red blood cells (RBCs), platelets, or white blood cells (WBCs), respectively. Pallor, fatigue, and dyspnea on exertion are common. Leukemic infiltration of various tissues, including the liver (hepatomegaly), spleen

(splenomegaly), skin (leukemia cutis), LNs (lymphadenopathy), bone (bone pain), gingiva, and central nervous system (CNS), can produce a variety of other symptoms. Hyperleukocytosis (more than100,000 WBCs per cubic millimeter) can lead to symptoms of leukostasis, such as ocular and cerebrovascular dysfunction or bleeding. [6]

## Classification

Over the past 3 decades, the classification system for AML has evolved from the French American British (FAB) (1976) system to the system developed by the World health organization (WHO) (2001 edition then 2008 edition).[13]

French American British (FAB) system has relied on morphology to separate AML from ALL and to categorize the disease based on degree of myeloid and monocytic differentiation, and on cytochemical stains, including myeloperoxidase (MPO) and Sudan Black B (SBB) for granulocytic differentiation and nonspecific esterase (NSE) for monocytic differentiation.[14]

In 2001, the WHO developed a newer classification system, which incorporates information from cytogenetics and evidence of dysplasia to refine prognostic subgroups that may define treatment strategies. They has been oriented towards categorization of disease entities according to underlying genetic alterations as they are usually associated with distinctive clinico-pathological features and may serve as specific diagnostic and prognostic markers. This orientation first manifested with the decision to create a category of "AML with recurrent genetic abnormalities".[14]

During this transition from the FAB system to the WHO classification, the percent blasts threshold for defining AML was lowered. The FAB classification (1976) had set the threshold for AML at 30% blasts, whereas the WHO classification lowered the threshold for diagnosing AML to 20% or more blasts. In addition, the WHO classification system allows AML to be diagnosed regardless of the percentage of marrow blasts in patients with abnormal hematopoiesis and characteristic clonal structural cytogenetic abnormalities with t(15;17), t(8;21), and inv. (16) or t(16;16).[13]

In 2003, the International Working Group for the Diagnosis and Standardization of Response Criteria accepted the cytochemical and immunophenotypic criteria of WHO as the standard for diagnosing AML, including the reporting of dysplasia according to morphology.[15]

In 2008, the WHO revised the diagnostic and response criteria for AML to include additional recurrent genetic abnormalities created by reciprocal translocations/inversions, and a new provisional category for some of the molecular markers that have been found to have a prognostic impact.[13]

### 1- FAB (French –American –Brititish) Classification

The most commonly used method of classification is that developed by the French-American-British (FAB) cooperative group, which divides AML into eight distinct subtypes (Tabel 2, Fig 1) that differ with respect to the particular myeloid lineage involved and the degree of leukemic cell differentiation. This distinction is based on the morphologic appearance of the blasts and their reactivity with histochemical stains, including MPO, SBB, and NSE; α naphthylacetate esterase (ANAE) and α naphthylbutyrate.[6, 16]

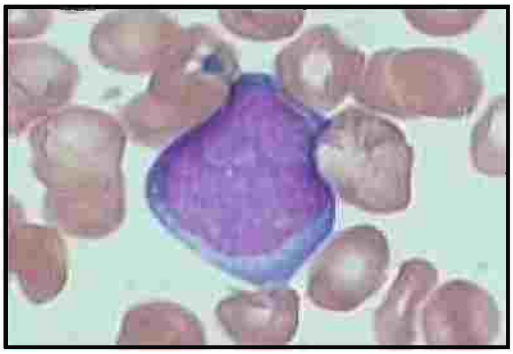
The major advantage of the FAB, lineage based, classification system is its ease of use. The cytological criteria are well defined; they do not require high technology and can be applied in most laboratories throughout the world. The classification is also applicable to the majority of cases of AML.[17]

Unfortunately, there is a general lack of clinical and biological relevance to this long-standing approach, e.g., failure to predict outcome, failure to predict response to particular therapeutic approaches and partial and imprecise correlation with cytogenetics. [18]

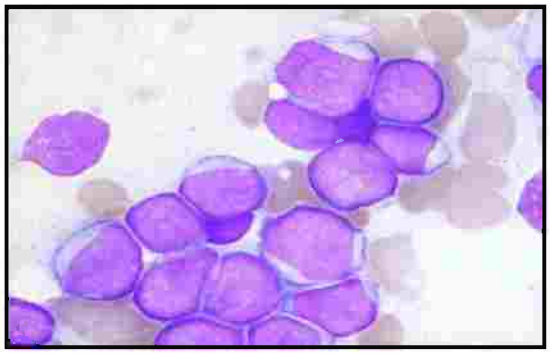
This approach remains a useful shorthand descriptor of myeloblast morphology, but should not be used alone as a classification for AML.[18]

**Table (2): FAB classification of AML (1976).[16, 19, 20]**

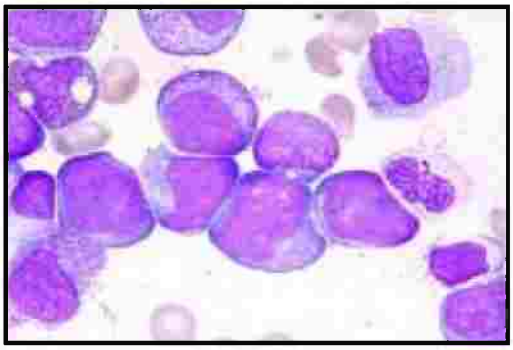
| FAB Subtype | Description |
|---|---|
| M0 (Acute myeloid leukemia minimally differentiated) | More than 30% blasts. Less than 3% blasts reactive for MPO, SBB or NSE |
| M1 (Acute myeloid leukemia without maturation) | More than 30% blasts. More than 3% blasts reactive for MPO or SBB. Less than 10% of marrow nucleated cells are promyelocytes or more mature neutrophils (<10 % granulocytic differentiation) |
| M2 (Acute myeloid leukemia with maturation) | More than 30% blasts. More than 3% blasts reactive for MPO or SBB. More than 10% of marrow nucleated cells are promyelocytes or more mature neutrophils (>10 % differentiating granulocytes) |
| M3 (Acute promyelocytic leukemia) | More than 30% blasts. Intense MPO and SBB. Promyelocytes and blasts with multiple Auer rods (faggot cells).<br>(a) Hypergranular promyelocytes with numerous Auer rods on Leishman stain<br>(b) A variant form showing cells with bilobed, multilobed or reniform nuclei (NSE-negative) and relative scarcity of hypergranular promyelocytes or of primitive cells with multiple Auer rods. |
| M4 (Acute myelomonocytic leukemia) | More than 30% myeloblasts, monoblasts and promonocytes >20% but <80 % , neutrophils and its precursors >20% , monocytic cells reactive for NSE. |
| M5a (Acute monoblastic leukemia without maturation) | More than 80% monocytic cells; monoblasts more than 80% of monocytic cells.<br>Monoblasts and promonocytes are NSE positive; monoblasts are MPO and SBB negative (may be positive in minority of cases) |
| M5b (Acute monocytic leukemia) | More than 80% monocytic cells; monoblasts less than 80% of monocytic cells; promonocytes predominate. Monoblasts and promonocytes are NSE positive; promonocytes have some scattered MPO and SBB positive granules. |
| M6 (Erythroleukemia) | More than 50% erythroid precursors and more than 30% of nonerythroid precursors are myeloblasts; Auer rods may be present in myeloblast. Dyserythropoiesis may be present. Erythroid precursors are Periodic Acid Schiff (PAS) positive. |
| M7 (Acute Megakaryoblastic leukemia) | Blasts are more than 30% of BM nucleated cells; blasts demonstrated to be megakaryoblasts by immunological markers and ultrastructural examination. |



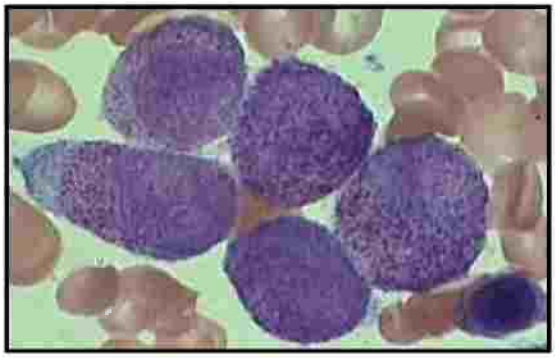
**AML M0**



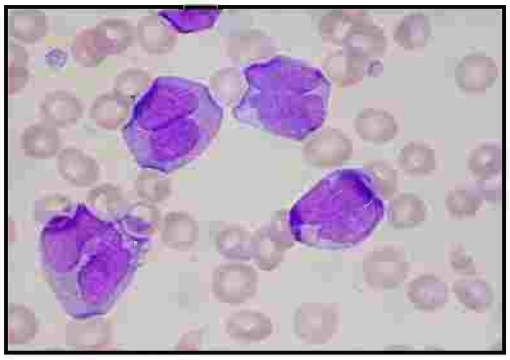
**AML M1**



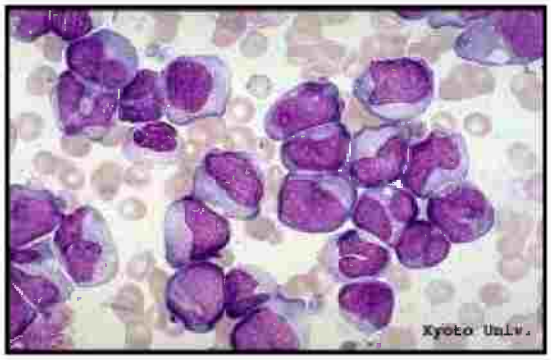
**AML M2**



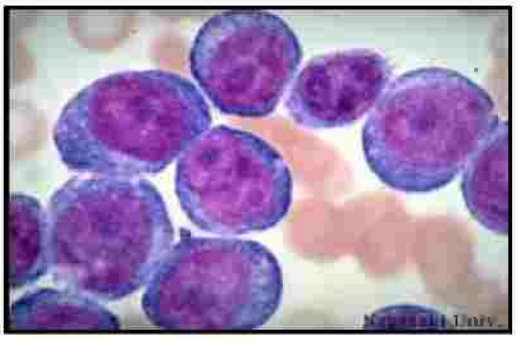
**AML M3**



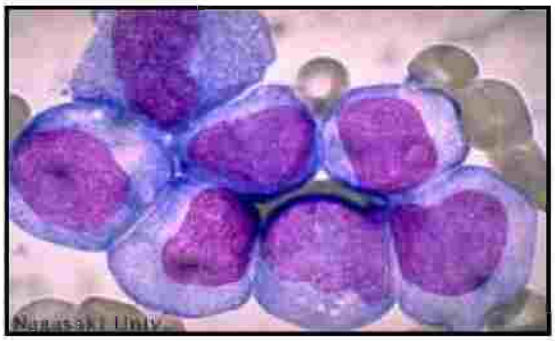
**AML M3v**



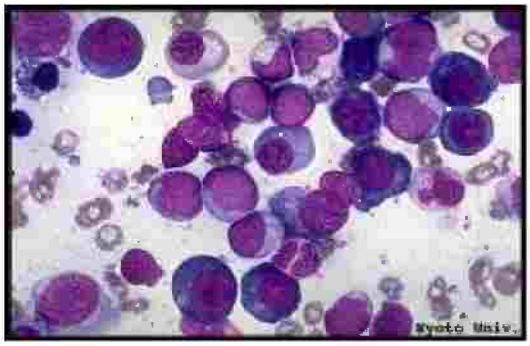

**AML M4**



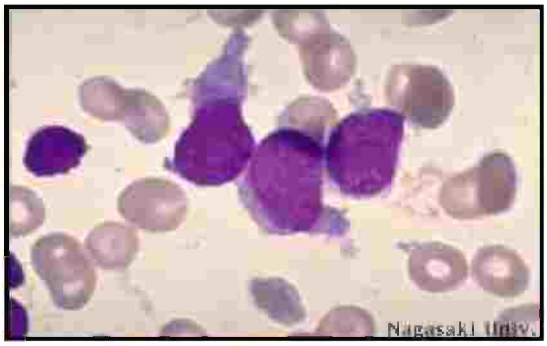
**AML M5a**



**AML M5b**




**AML M6**




**AML M7**

**Figure (1):** AML morphology (FAB classification).[21-23]

## 2- WHO Classification of AML (2008)

The WHO classification (Table 3, Fig 2) uses all available information (morphology, cytochemistry, immunophenotyping, genetics and clinical features) to define clinically significant disease entities and to provide a classification that can be used in daily clinical practice as well as to serve as a common language for clinical trials and laboratory investigations.[24]

In the WHO classification, the term "myeloid" includes all cells belonging to the granulocytic (neutrophil, eosinophil, basophil), monocytic/macrophage, erythroid, megakaryocytic and mast cell lineages. The WHO criteria for myeloid neoplasms apply to initial diagnostic PB and BM specimens obtained prior to any definitive therapy for a suspected hematologic neoplasm. Morphologic, cytochemical, and/or immunophenotypic features are used for establishing the lineage of the neoplastic cells and for assessment of their maturation.[24]

A major departure by the WHO from the FAB criteria was to lower the threshold for the diagnosis of AML from 30% to 20% blasts in the PB and/or the BM aspirate. Exceptions include AML with t(8;21), inv(16), or t(15;17), in which the diagnosis of AML is made regardless of the percentage of BM blasts.[15]

The 20% blast threshold is not a mandate to treat the patient as having AML or blast transformation; therapeutic decisions must always be based on the clinical situation after all information is considered. A tumoral proliferation of blasts in an extramedullary site (myeloid sarcoma) is also considered to be AML when it is found de novo or in a patient with myelodysplastic syndrom (MDS) or myelodysplatic syndrom/myeloproliferative neoplasms (MDS/MPNs), and blast transformation in cases of myeloproliferative neoplasms (MPNs).[24]

**Table (3): Acute myeloid leukemia and related precursor neoplasms (WHO 2008).**[25]

| |
|---|
| **AML with recurrent genetic abnormalities** |
| • AML with t(8;21)(q22;q22), *(RUNX1T1;RUNX1)*<br>• AML with abnormal bone marrow eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22), (*CBFB;MYH11*)<br>• Acute promyelocytic leukemia with t(15;17)(q22;q11–21), (PML;RARA)<br>• AML with 11q23 (MLL) abnormalities<br>• AMl with t(6,9) (p23;q34)<br>• AML inv (3)(q21q26.2)or t(3,3)(q21;q26.2)<br>• AML(megakaryoblastic)with t(1;22)(p13;p13)<br>• Provisional entity*: AML with mutated NPM1<br>• Provisional entity*: AML with mutated CEBPA |
| **AML with myelodysplasia related changes** |
| • Following a myelodysplastic syndrome or (MDS)/myeloproliferativeneoplasm (MPN)<br>• Without antecedent MDS or MDS/MPN, but with dysplasia in at least 50% of cells in two or more myeloid lineages |
| **AML and myelodysplastic syndromes, therapy related** |
| Cytotoxic agents implicated in therapy-related hematologic neoplasms:<br>- alkylating agents;<br>- ionizing radiation therapy;<br>- topoisomerase II inhibitors;<br>- other types. |
| **AML, Not otherwise specified** |
| • AML with minimal differentiation<br>• AML without maturation<br>• AML with maturation<br>• Acute myelomonocytic leukemia<br>• Acute monoblastic/monocytic leukemia<br>• Acute erythroid leukemia<br>- Pure erythroid leukemia<br>- Erythroleukemia, erythroid/myeloid<br>• Acute megakaryoblastic leukemia<br>• Acute basophilic leukemia<br>• Acute panmyelosis with myelofibrosis (syn.: acute myelofibrosis; acute myelosclerosis) |
| **Myeloid sarcoma (syn.: extramedullary myeloid tumor; granulocytic sarcoma; chloroma)** |
| **Myeloid proliferations related to Down syndrome** |
| - Transient abnormal myelopoiesis (syn.: transient myeloproliferative disorder)<br>- Myeloid leukemia associated with Down syndrome |
| **Blastic plasmacytoid dendritic cell neoplasms** |

*Provisional entity: newly described or characterized disorders that are clinically and/or scientifically important and should be considered in the classification, but for which additional studies are needed to clarify their significance and will be incorporated as confirmed entities in the next revision process. CEBPα: CCAAT/enhancer binding protein alpha gene, inv: inversion, NPM1: nucleophosmin gene, p: short arm of chromosome, q: long arm of chromosome, t: translocation.

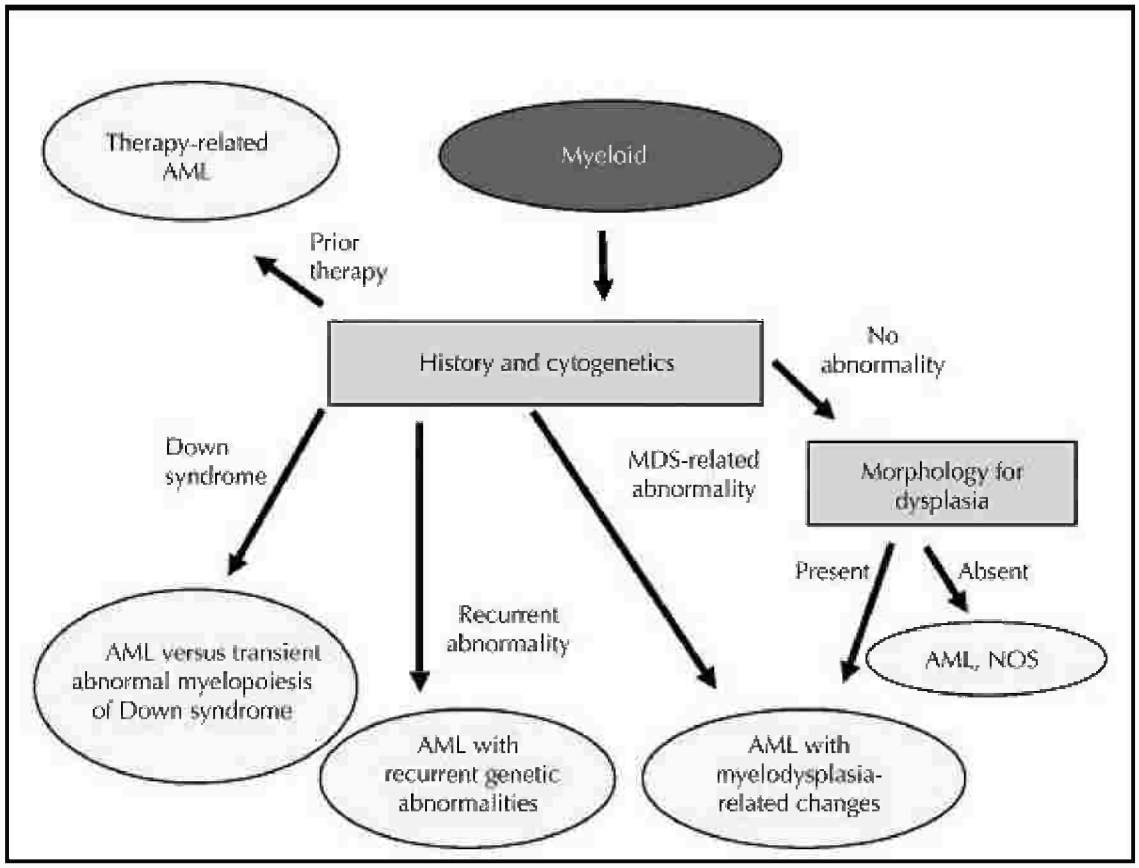



**Figure (2):** Schematic representation of algorithmic approach to diagnose AML.

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome;

NOS: not otherwise specified.[26]

## Diagnosis of AML

## 1- Morphology

Examination of BM and PB (Fig 3) specimens provides a rapid initial and frequently conclusive diagnosis. A bone marrow aspirate is part of the routine diagnostic work-up of a patient with suspected AML. The panel considers a marrow trephine biopsy optional, but it should be performed in patients with a dry tap (punctio sicca). Blood and marrow smears are morphologically examined using Leishman stain.[25]

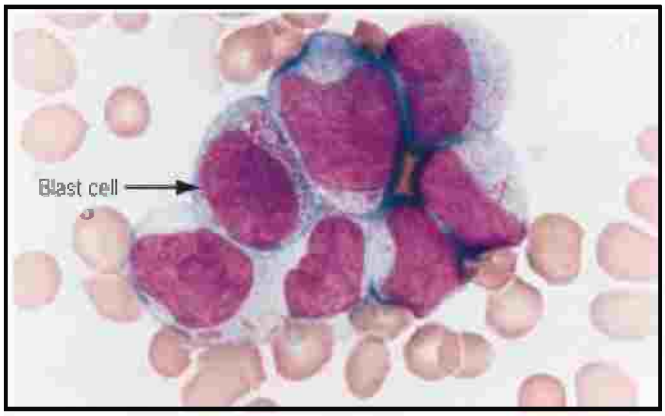



**Figure (3):** Light microscopy of peripheral blood showing numerous myeloblasts and marked thrombocytopenia.[27]

Although a BM core biopsy (Fig 4) may not be required in every case, an adequate biopsy does provide the most accurate assessment of the marrow cellularity, topography, stromal changes, and maturation pattern of the hematopoietic lineages.[24]

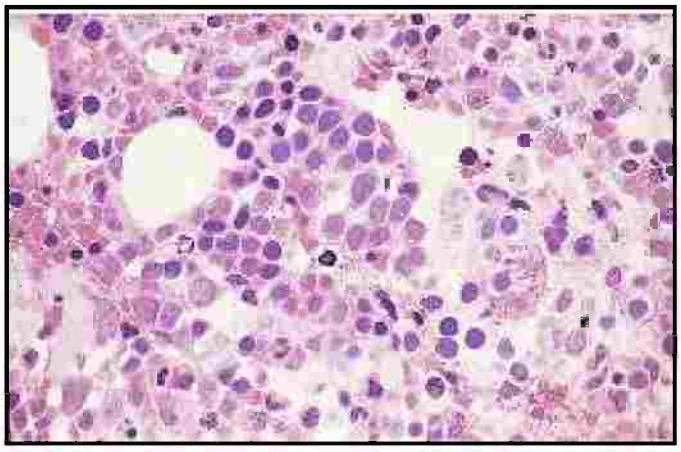


**Figure (4):** Bone marrow trephine biopsy of a patient with AML showing sheets of myeloblasts.[28]

In establishing a diagnosis of AML, it is critical to obtain an accurate blast count performed on at least 500 nucleated BM cells and at least 200 PB leukocytes, with the earlier containg spicules. In instances of a 'dry tap', it is acceptable to perform a myeloblast count on an air-dried touch preparation. [29]

For a diagnosis of AML, a marrow or blood blast count of 20% or more is required. However, there are several exceptions to the requirement of 20% myeloblasts in blood or BM to establisha diagnosis of AML:

- In cases of the cytogenetic abnormalities t(15;17), inv(16)/t(16;16), or t(8;21), a diagnosis of AML can be made irrespective of blast count.
- In cases of monocytic differentiation, promonocytes (primitive monocytic cells with features intermediate between monocytes and monoblasts) are included along with blasts in the blast count.
- In cases where erythroid elements comprise at least 50% of the BM nucleated cells and blasts comprise ≥ 20% of the nonerythroid cells, a diagnosis of acute erythroleukemia (erythroid-myeloid subtype) is made.

- In cases of pure erythroid leukemia, undifferentiated erythroblasts replace the marrow and comprise ≥ 80% of the marrow cells, even though myeloblasts are not increased.[25, 29]

## 2- Cytochemistry

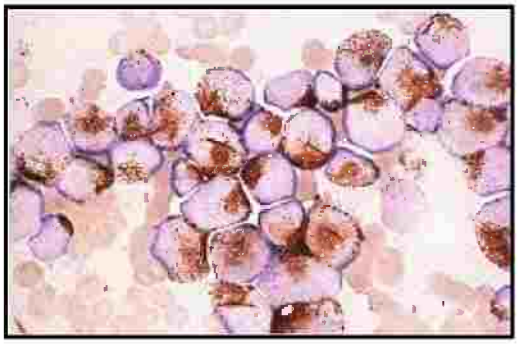
To identify lineage involvement, some countries still rely more on cytochemistry (Fig 5), rather than on immunophenotyping (usually by flow cytometry), using MPO or SBB and NSE stains.[25]

Detection of MPO (if present in ≥ 3% of blasts) indicates myeloid differentiation, but its absence does not exclude a myeloid lineage because early myeloblasts and monoblasts may lack MPO. Sudan black B staining parallels MPO but is less specific.[25]

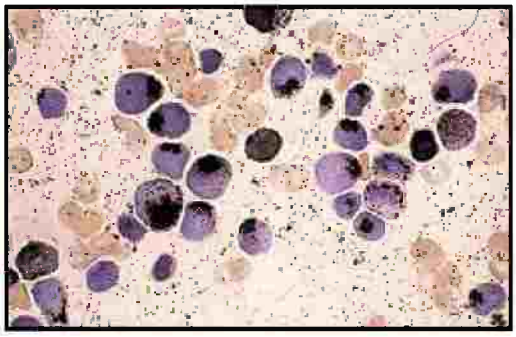
Nonspecific esterase and α naphthyl acetate esterase stains show diffuse cytoplasmic activity in monoblasts (usually > 80% positive) and monocytes (usually >20% positive).[25]

In acute erythroid leukemia, a Periodic acid–Schiff stain may show large globules of PAS positivity.[25]

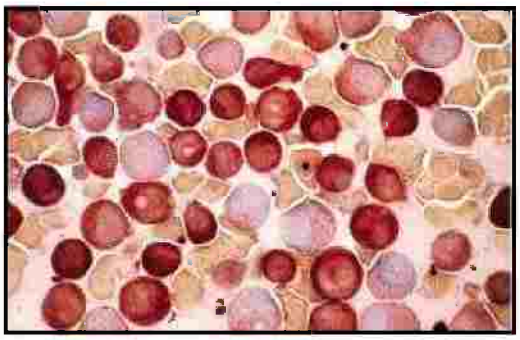
Iron stains may allow for the detection of iron stores, normal sideroblasts, and ring sideroblasts.[25]



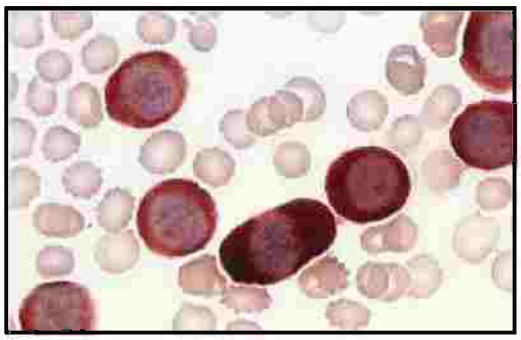
**1- MPO**



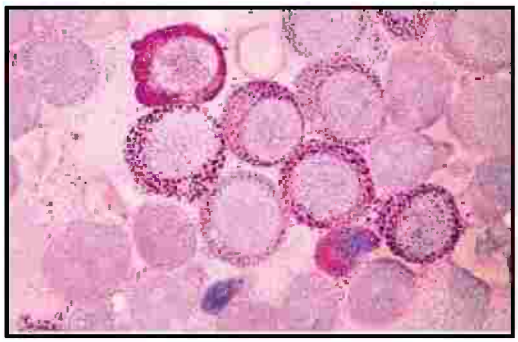
**2- SBB**



**3- NSE**



**4- ANAE**



**5- PAS**

**Figure (5):** Cytochemical stains: Different cytochemical stains for diagnosis and differntiation of AML. 1- Cytochemical stain for MPO demonstrating numerous MPO positive cells, 2- Cytochemical stains for SSB demonstrates dense, darkly stained cytoplasmic granules in myeloid cells 3- NSE reactione, approximately 85 % of the cells are strongly and diffusely positive, 4- ANAE shows strong diffuse esterase activity in the cytoplasm of the blasts, 5- Intense granular and diffuse PAS reaction in the BM of a patient with M6.[30]

## 3- Immunophenotyping

Immunophenotyping (Fig 6) using multiparameter (commonly at least 3 to 4 color) flow cytometry is used to determine lineage involvement of a newly diagnosed acute leukemia (Table 4).[31]

There is no general consensus on the cutoff point for considering an acute leukemia to be positive for a marker. For most markers, a commonly used criterion is 20% or more of leukemic cells expressing the marker, whereas for selected markers (eg, cytoplasmic CD3, MPO, TdT, CD34, CD117) a lower cutoff has been applied (10%).

Quantification of expression patterns of several surface and cytoplasmic antigens is necessary for lineage assignment, to diagnos MPAL, and to detect aberrant immunophenotypes allowing for measurement of minimal residual disease (MRD).

Flow cytometric determination of blast count should not be used as a substitute for morphologic evaluation.[32]

Immunophenotyping is required to establish the diagnosis of AML with minimal differentiation, acute megakaryoblastic leukemia, and acute leukemias of ambiguous lineage.[33]

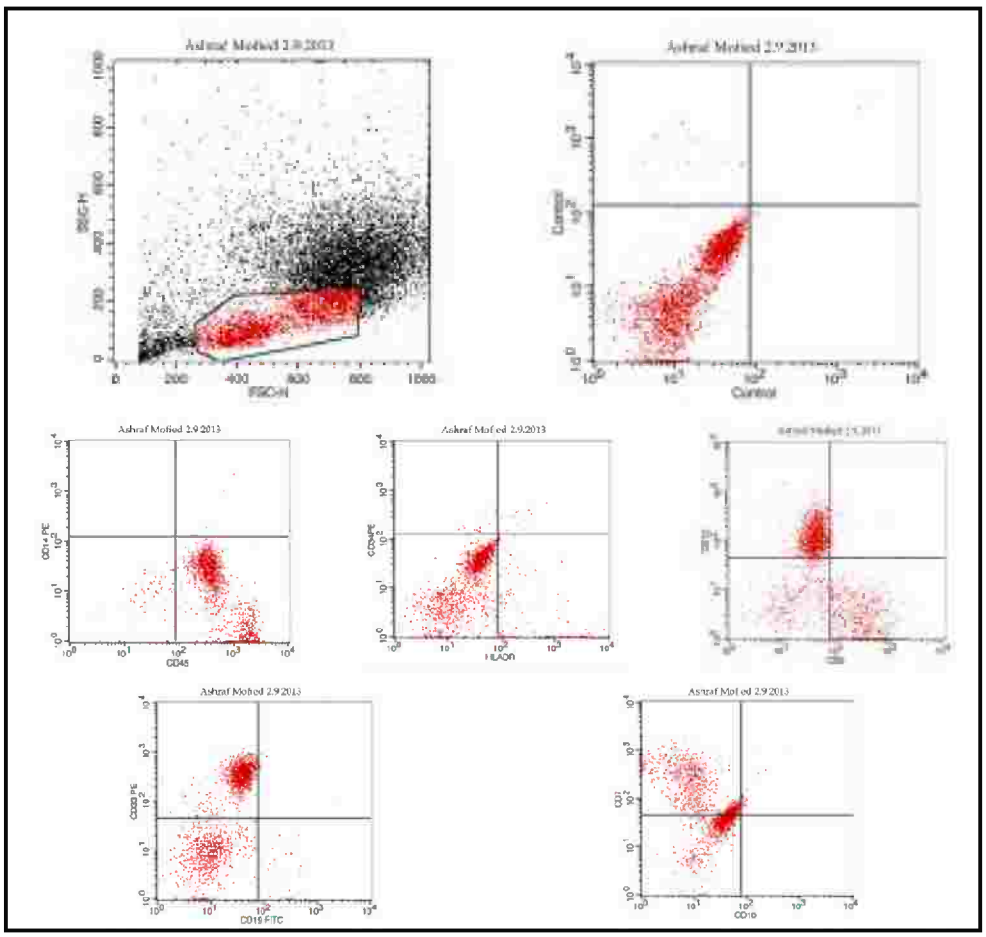


**Figure (6):** Scatter plots of immunophenotyping - the gated population of neoplastic cells showed positivity for CD45, CD13, CD33, CD7 and negativity for HLADR, CD34, CD10, CD19, CD5, and CD14.

AML with minimal differentiation is an AML without morphologic and cytochemical evidence of myeloid differentiation. Most cases express early hematopoiesis-associated antigens (eg, CD34, CD38, and HLA-DR) and lack most markers of myeloid and monocytic maturation; while MPO is negative by cytochemistry, detection of intracytoplasmic MPO antigens may be positive by flow cytometry in at least a fraction of blasts. Acute megakaryoblastic leukemia is a leukemia with 20% or more blasts of which 50% or more are of megakaryocytic lineage; megakaryoblasts typically express one or more of the platelet glycoproteins CD41 and/or CD61, and less commonly CD42. Acute leukemias of ambiguous lineage are rare leukemias and comprise those cases that show no evidence of lineage differentiation (ie, acute undifferentiated leukemia [AUL]) or those with blasts that express markers of more than one lineage (ie, MPAL).[25]

Some AMLs with recurrent genetic abnormalities are associated with characteristic immunophenotypic features. For example, AMLs with t(8;21) frequently express the lymphoid markers CD19 or, to a lesser extent, CD7; they may also express CD56; AMLs with inv(16) frequently express the T lineage–associated marker CD2; and AMLs with NPM1 mutation typically have high CD33 but absent or low CD34 expression.[34]

**Table (4): Expression of cell-surface and cytoplasmic markers for the diagnosis of AML and MPAL.[25]**

| **EXPRESSION OF MARKERS FOR DIGNOSIS** | |
|---|---|
| **Diagnosis of AML*** | |
| Precursor stage | CD34, CD38, CD117, CD133, HLA-DR |
| Granulocytic markers | CD13, CD15, CD16, CD33, CD65, cMPO |
| Monocytic markers | NSE, CD11c, CD14, CD64, lysozyme, CD4, CD11b, CD36, NG2 homologue |
| Megakaryocytic markers | CD41 (glycoprotein IIb), CD61 (glycoprotein IIIa), CD42 (glycoprotein Ib) |
| Erytheroid marker | CD235a (glycophorin A) |
| **Diagnosis of MPAL** | |
| Myeloid lineage | MPO or evidence of monocytic differntiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme) |
| B-lineage | CD 19 (strong) with at least one of the following: CD79a, CD10, cCD22, or CD19 (weak) with at least 2 of the following: cCD22, CD10 |
| T-lineage | cCD3 or sufrace CD3 |

* For the diagnosis of AML, the table provides a list of sletected markers rather than a mandatory marker panel. cMPO: cytoplasmic myeloperoxidase, NSE: nonspecific esterase.

## 4- Cytogenetics

Conventional cytogenetic analysis is a mandatory component in the diagnostic evaluation of a patient with suspected acute leukemia. Chromosomal abnormalities are detected in approximately 55% of adult AML cases.[35]

Cytogenetics confer the most important prognostic information in AML (Table 5), along with patient's age, performance status, presenting WBC counts, FLT-3 mutation, MLL partial tandem duplication, and whether a patient has de novo or secondary disease. Therefore, detailed karyotypes should be performed on all patients with AML at diagnosis.[36]

In addition, because specific, recurrent cytogenetic abnormalities confer varying prognosis and differing responses to chemotherapy, or transplantation regimens, so they will be increasingly used to direct patients to different targeted therapies. It is critical to report the specific chromosome abnormality, rather than reporting the broader prognostic categorizations frequently employed (eg, normal or abnormal, favorable or unfavorable).[15]

Seven recurrent balanced translocations and inversions, and their variants, are recognized in the WHO category "AML with recurrent genetic abnormalities." Furthermore, several cytogenetic abnormalities are considered sufficient to establish the WHO diagnosis of "AML with myelodysplasia-related features" when 20% or more blood or marrow blasts are present (Fig 7& 8) . A minimum of 20 metaphase cells analyzed from BM is considered mandatory to establish the diagnosis of a normal karyotype, and to define an abnormal karyotype. Abnormal karyotypes may be diagnosed from blood specimens.[25]

**Table (5): Common chromosome aberrations in AML.[1]**

| Translocations/inversions | Genes | Morphological associations | Incidence* |
|---|---|---|---|
| t(8;21)(q22;q22) | RUNX1; RUNX1T1 | M2 with Auer rods | 6% |
| inv(16)(p13q22)or t(16;16)(p13;q22) | CBFβ;MYH11 | M4Eo | 7% |
| t(15;17)(q22;q11–21) | PML;RARA | M3, M3V | 7% |
| t(9;11)(p22;q23) | MLL;AF9 | M5 | 1% |
| t(6;11)(q27;q23) | MLL;AF6 | M4, M5 | 1% |
| inv(3)(q21q26)or t(3;3)(q21;q26) | EVI1;RPN1 | M1, M4, M6, M7 | 1% |
| t(6;9)(p23;q34) | DEK;NUP214 | M2, M4 | 1% |

* Determined among 1311 patients with de-novo AML enrolled into a study of 8461 cases of the Cancer and Leukemia.[37]

Cytogenetic studies define 3 general risk groups: favorable, intermediate, and adverse. The favorable-risk group includes acute promyelocytic leukemia with t(15;17) and is the only AML subtype that is associated with a specific therapy (ie, trans-retinoic acid). Acute myeloid leukemias with t(8;21) or inv(16)/t(16;16) tend to be in the favorable-risk category but they are treated with the same agents (cytarabine, doxyrubicin) as all the other subtypes. Although this type of leukemia is considered ''favorable,'' half of the patients still die from their disease, and the results are even worse in older patients.[26]

Cases with t(9;11) are associated with intermediate risk, along with cases showing gains of whole chromosomes or loss of the Y chromosome.[26]

The adverse risk group includes t(6;9), inv(3)/t(3;3), and complex karyotype (3 or more cytogenetic abnormalities). Cases with multiple alterations tend to be observed with older patients and have an extremely poor prognosis, with patients rarely surviving beyond 1 year.

Until recently, it has been difficult to stratify the subset of AMLs (40%) with normal cytogenetics (CN-AML). The overall risk in this group is ''intermediate''; however, the outcome in individual cases varies considerably and is related, in part, to the presence or absence of specific molecular abnormalities.[26]

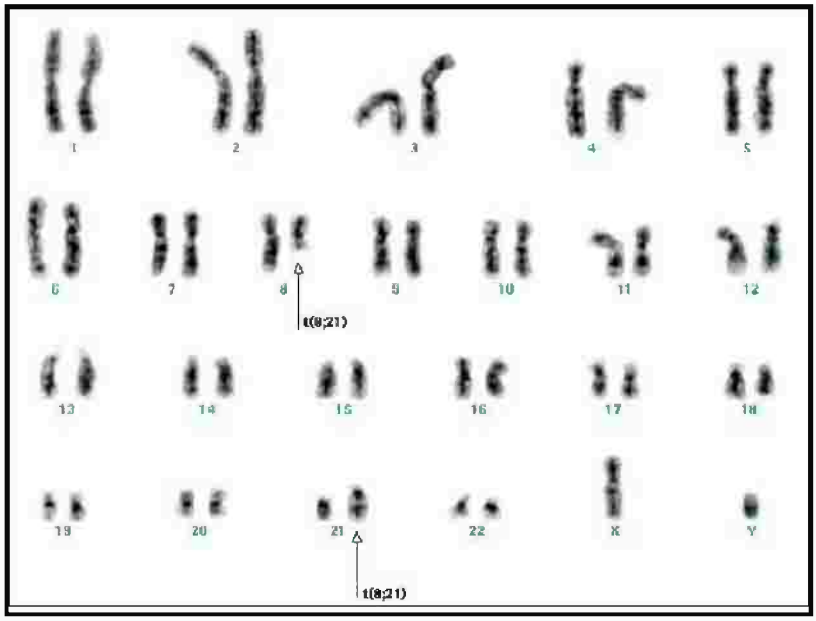



**Figure (7):** Conventional cytogenetics, G-banded karyotype, showing t(8;21).[38]

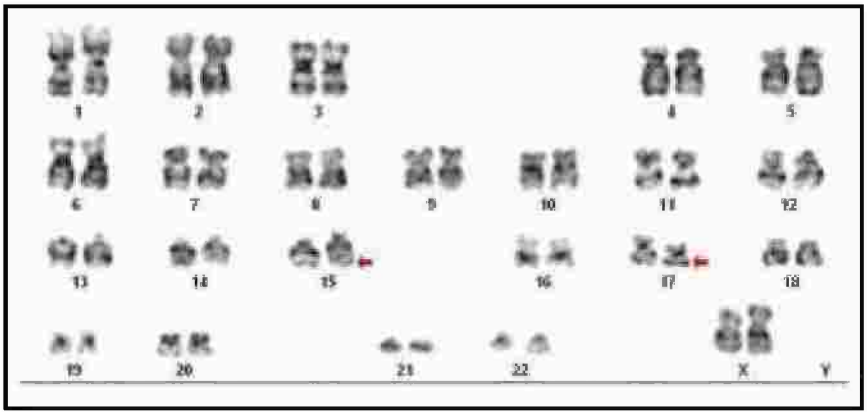


**Figure (8):** Conventional cytogenetics, G-banded karyotype, showing t(15;17).[39]

## 5- Molecular Genetics

Molecular diagnostic tools such as fluorescent in situ hybridization (FISH) (Fig9), Southern blotting, and Real Time PCR (Q-PCR), are more specific and/or sensitive than classic cytogenetics. It requires focus on specific genes for testing rather than providing a screening tool for multiple abnormalities such as RUNX1-RUNX1T1, CBFB-MYH11, and MLLT3-MLL.[18]

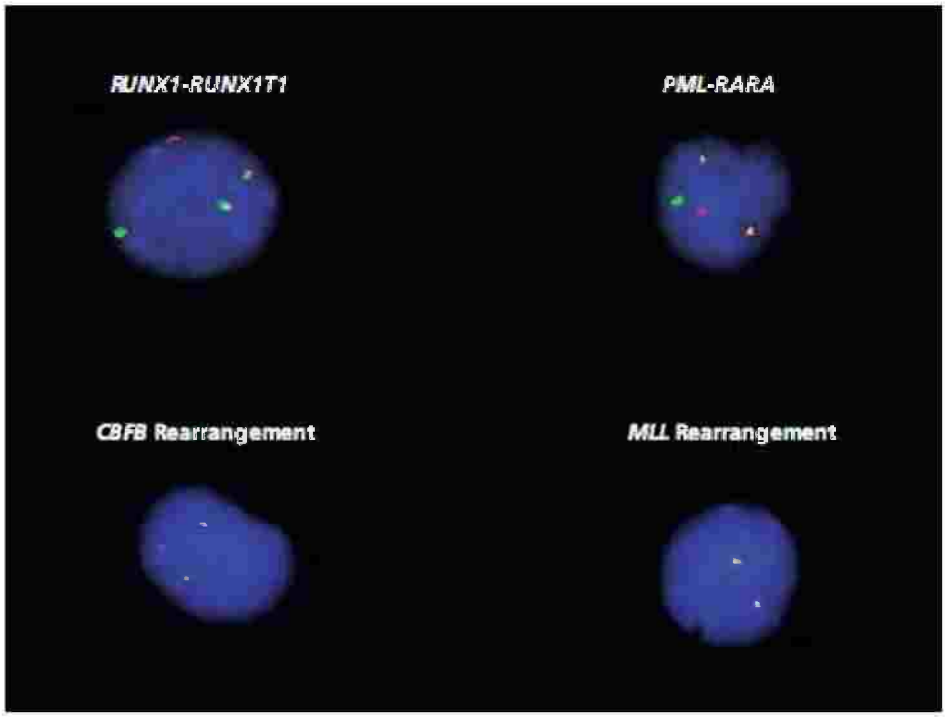



**Figure (9):** Fluorescence in situ hybridization in four different patients with AML showing four major cytogenetic/genetic categories of AML: RUNX1 – RUNX1T fusion indicative of t(8;21) (top left); PML – RARA fusion indicative of t(15;17) (top right); CBFβ rearrangement, which is likely to indicate CBFβ – MYH11 (bottom left); MLL rearrangement (bottom right).[40]

In recent years, gene mutations and deregulated expression of genes and non coding RNAs (eg, microRNAs) have been identified (Table 6), providing insights into the mechanisms of leukemogenesis and unraveling the enormous molecular genetic heterogeneity within distinct cytogenetically defined subsets of AML, in particular the large group of (CN-AML).[41-44]

With progress in genomics technology, such as gene and microRNA-expression profiling, genome-wide single nucleotide polymorphism (SNP) –based mapping arrays, next-generation sequencing (NGS) techniques and functional genomics, systematic characterization of cancer genomes have now become feasible, and the identification of disease-relevant mutations and related gene and microRNA profiles will be facilitated in the future.

From a clinical perspective, there are at least three important aspects with respect to the genetic changes in AML.[25] First, the current WHO classification reflects the fact that an increasing number of cases of AML can be categorized on the basis of their underlying genetic defects that define distinct clinicopathologic entities.[33] Second, it has become clear that specific chromosomal abnormalities and molecular genetic (Fig 10) changes are

among the most important prognostic markers and therefore may be used for stratification of patients with AML to risk-adapted therapeutic strategies. Finally, novel therapies are being developed that target some of the identified genetic defects. It is therefore anticipated that these genetic markers will acquire a predictive value, that is, the ability to predict differential efficacy of therapy. Thus, it is hoped that genetic markers will allow us to optimize the treatment of distinct subtypes of AML.[45]

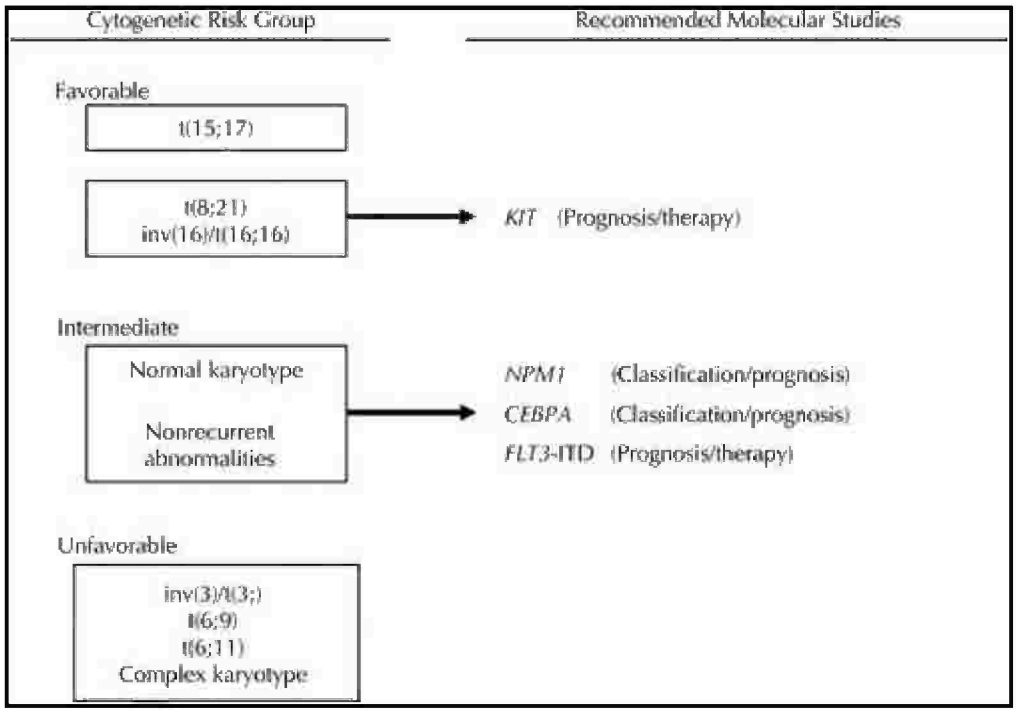



**Figure (10):** Recommended molecular studies for AML classification and risk stratification. FLT3: fms-related tyrosine kinase 3, ITD: internal tandem duplication, NPM1: (nucleophosmin (nucleolar phosphoprotein B23, numatrin)), CEBPA: CCAAT/enhancer-binding protein alpha.[26]

**Table (6): Biological and clinical features associated with gene mutations in AML.[44]**

| Gene | Biological / Clinical Features |
|---|---|
| NPM1 | Encodes a phosphoprotein with pleiotropic functions. NPM1 mutations found in 25-35% of all adult AML, in 45-64% of CN- AML, in 35-40% of AML with 9q deletion, and in about 15% of AML with trisomy 8; NPM1 mutations associated with FLT3-ITD (~40%) and FLT3 TKD mutations. Association with female sex, higher BM blast counts and serum LDH level , myelomonocytic or monocytic morphology, and high CD33 and absent CD34 expression. NPM1 mutation is associated with better response to induction chemotherapy; genotype "mutant NPM1 without FLT3-ITD" is associated with favorable RFS and OS. |
| CEBPα | Encodes a master regulatory transcription factor in hematopoiesis. CEBPA mutations found in 10-18% of CN- AML, and in about 40% of AML with 9q deletion occurring within a non-complex karyotype. CEBPA mutations associated with higher CR rate and better RFS and OS. |
| MLL | Encodes a DNA binding protein which regulates gene expression in hematopoiesis possibly through epigenetic mechanisms. MLL-PTD found in 5-11% of CN-AML, and up to 90% of AML with trisomy 11. Associated with shorter CR duration and inferior RFS and EFS ; autologous transplantation may improve outcome. |
| RUNX1 | Encodes a transcription factor involved in normal hematopoietic differentiation. RUNX1 mutations found in about 10% of CN-AML, nearly all AML with trisomy 13, and about half of AML with trisomy 21. Data on prognostic significance is not yet available. |
| FLT3 | Member of the class III receptor tyrosine kinase family; FLT3 and its ligand play an important role in proliferation, survival and differentiation of hematopoietic progenitor cells. |
| ITD | FLT3-ITD is found in about 20% of all AML, 28-34% of CN- AML and is associated with inferior outcome; level of mutant allele likely of importance; homozygous FLT3 mutations as a result of mitotic recombination leading to partial UPD. |
| TKD | FLT3 TKD point mutations are found in 5-10% of all AML, in 11-14% of CN-AML, and in 14-24% of AML with inv(16). Prognostic significance controversial. |
| KIT | Member of the class III receptor tyrosine kinase family; KIT and its ligand stem cell factor have a key role in survival, proliferation, differentiation, and functional activation of hematopoietic progenitor cells. KIT mutations found in about 30% of CBF AML, and in rare cases of other AML types.<br>KIT mutations, in particular in exon 17, associated with inferior outcome in many but not all studies. |
| RAS | Encodes membrane-associated proteins regulating mechanism of proliferation, differentiation and apoptosis. NRAS mutations found in 9-14% of CN-AML, in up to 40% of CBF AML [in particular inv(16)], and in 25% of AML with inv(3); KRAS mutations found in 5-17% of CBF AML. So far, no prognostic significance found; mutant NRAS may confer sensitivity to cytarabine. |
| WT1 | Encodes a transcription factor implicated in regulation of apoptosis , proliferation, and differentiation of hematopoietic progenitor cells. Mutations found in about 10% of CN-AML. Initial studies suggest association with induction failure, particularly in patients with WT1 mutation and concurrent FLT3-ITD; prognostic impact under investigation. |

CN: cytogenetically normal; LDH: lactate dehydrogenase; RFS: relapse-free survival; OS: overall survival; CR: complete remission; EFS: event-free survival. All mentiond genes are listed in abbreviation list.

## Prognosis

A number of clinical and biologic features that reflect the heterogeneity of AML are used to predict the likelihood that a patient will have a response to treatment.[6]

Prognostic factors may be subdivided into those related to patient characteristics and general health condition and those related to characteristics particular to the AML clone. The former subset usually predicts treatment-related mortality (TRM) and becomes more important as patients' age increases while the latter predicts resistance to, at least, conventional therapy.

### 1- Patient-Related Prognostic Factors

Increasing age is an adverse prognostic factor.[10, 46] Older patients have worse outcomes than younger patients. For those patients less than 60 years, complete remission (CR) rates of 75% and 5 year survival rates of 35-40% can be expected. Once the patient age exceeds 60 years, CR rates fall to 45-55% and 5 year survival rates fall to less than 10%. The reasons for this include a reduction in the capacity of the patient to tolerate intensive therapy as well as a change in the nature of the disease to one characterized by a higher frequency of adverse cytogenetic, CD34 positivity and MDR-1 expression.[46-48]

### 2- AML-Related Prognostic Factors

- White blood cell count.
- Existence of prior MDS.
- Previous cytotoxic therapy for another disorder .
- Cytogenetics : The karyotype of the leukemic cells is the strongest prognostic factor for response to induction therapy and for survival as follow:[41, 49]

- 25% of patients will have favorable cytogenetics which are more common in younger patients and in de-novo AML and are associated with CR rates of over 90% and a 5 year survival of around 65%.
- 10% of patients will have adverse cytogenetics which tend to be older, often with prior history of myelodysplasia or exposure to chemotherapy and can expect CR rates of around 60% and a 5 years survival of 10%.
- The remaining 45-60% of patients will have intermediate cytogenetic prognosis. The majority of these will have a normal karyotype. The CR rates for this group are about 80% with 5 year survival of 30-40%.

- Molecular genetic changes in the leukemic cells at diagnosis (Table 7).
- Splenomegaly and elevated serum LDH levels.
- Monitoring of MRD: By real time-PCR detecting leukemia-specific targets, FISH, or by multiparameter flow cytometry identifying leukemia-associated aberrant phenotypes . Potentially useful applications of MRD monitoring include early assessment of response to therapy to improve risk stratification and guide post-remission therapy; and post-treatment monitoring to detect impending relapse and guide preemptive therapy.[50]
- Presence of extra-medullary or CNS disease.
- Response to therapy:

Response to induction therapy has long been recognized as a major independent prognostic factor in AML, predicting risk of relapse and overall survival, leading to the development of standardized response criteria.[51]

The time taken to clear leukemic blasts from the PB or the BM after 1-2 courses of induction chemotherapy is an important guide to outcome. The UK Medical Research Council (MRC) consider a blast count of greater than 15% after course 1, or greater than 5% after course 2, as indicative of poor prognosis. The German AML Cooperative Group (AMLCG) reports that failure to achieve blast clearance by day 16 is a poor prognostic marker.[52]

**Table (7): Standardized reporting for correlation of cytogenetic and molecular genetic data with prognosis in AML.[25]**

| Prognosis | Cytogenetic and molecular genetic data |
|---|---|
| Favorable | t(8;21)(q22;q22); RUNX1-RUNX1T1; inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFβ-MYH11<br>Mutated NPM1 without FLT3-ITD (normal karyotype)<br>Mutated CEBPα (normal karyotype) |
| Intermediate-I* | Mutated NPM1 and FLT3-ITD (normal karyotype)<br>Wild-type NPM1 and FLT3-ITD (normal karyotype)<br>Wild-type NPM1 without FLT3-ITD (normal karyotype) |
| Intermediate-II | t(9;11)(p22;q23); MLLT3-MLL; Cytogenetic abnormalities not classified as favorable or adverse |
| Adverse | inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1<br>t(6;9)(p23;q34); DEK-NUP214<br>t(v;11)(v;q23); MLL rearranged<br>-5 or del(5q); -7; abnl(17p); complex karyotype |

*Includes all AMLs with normal karyotype except for those included in the favorable subgroup; most of these cases are associated with poor prognosis, but they should be reported separately because of the potential different response to treatment.

ITD: internal tandem duplication; CBFβ: core-binding factor,beta subunit; MYH11: myosin, heavy chain 11, smooth muscle; NPM1: (nucleophosmin (nucleolar phosphoprotein B23, numatrin)); RUNX1: runt-related transcription factor 1; CEBPα: CCAAT/enhancer-binding protein alpha; MLL: myeloid/lymphoid or mixed-lineage leukemia; FLT3: fms-related tyrosine kinase 3;NUP214: nucleoporin 214kDa; RPN1: ribophorin I; EVI1: ecotropic viral integration site 1.

## Treatment

The primary objective in treating patients with AML is to induce remission and thereafter prevent relapse. Although obtaining a remission is the first step in controlling the disease, it is also important for patients to emerge from the induction phase in a condition to tolerate subsequent, more intensive treatments during consolidation to achieve durable disease control. Patients who do not receive post-remission therapy will experience relapse, usually within 6 to 9 months. Therapy for AML includes: supportive treatment, remission induction followed by continuation (post-remission therapy) (Fig 11 & 12).[13, 25, 53, 54]

**Supportive care**

It is unusual for induction chemotherapy not to clear most of the leukaemic blasts, however, this is at a cost of 3 – 4 weeks of severe pancytopenia. Supporting patients through the period of marrow suppression is crucial to treatment outcome; indeed many hold the view that the main reason that treatment has improved is due to improvements in supportive care. It is therefore important that patients are treated in an environment where all necessary supportive facilities are available. Several components of supportive care have to be in place during this period. Careful monitoring of biochemical parameters of renal and hepatic function and coagulation is required. Central venous access is now considered essential, together with high quality and readily available blood product support.[55]

A priority is the prevention and management of infection. Most patients will receive prophylactic oral antibiotics and antifungals to minimize the risk of infection during the neutropenic period, although routine use of the latter can still be debated. The risk of infection is related to the degree of neutropenia, which may be either a result of BM infiltration or cytotoxic-induced aplasia.[47, 56]

Since hospital-acquired infections are becoming an increasing problem, it can be safer for the patient to be at home provided that close monitoring can be undertaken in the day clinic and that rapid re-admission to specialist care is available.[55]

**Induction of Remission**

Induction chemotherapy is given to reduce the level of the leukemic burden below the level of detection and to restore normal hematopoiesis (Table 8).[57]

For 30 years, most patients with AML who have been treated have received remission induction therapy with what is commonly called "3+7" protocol, this is the backbone of treatment to induce remission.[58] Three days of an anthracycline (eg, daunorubicin, idarubicin, orthe anthracenedione mitoxantrone) that affects topoisomerase II and 7 days of cytarabine, ("3+ 7") currently remains the standard for induction therapy. With such regimens CR is achieved in 60% to 80% of younger adults. No other intervention has been convincingly shown to be better.[53, 59]

Induction chemotherapy should be started after the diagnostic work-up has been completed, preferably with minimal delay. Retrospective data suggest that treatment outcome might be adversely impacted when the time from diagnosis to start of treatment increases beyond 5 days.[60]

**Table (8): Response criteria in AML.**[15]

| Category | Definition |
|---|---|
| Morphologic leukemia-free state | Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required. |
| Complete remission (CR) | Bone marrow blasts <5%<br>Absence of blasts with Auer rods and absence of extramedullary disease<br>Absolute neutrophil count > 1.0X $10^3$/UL and platelet count > 100 X $10^3$/UL<br>Patient independent of red blood cell transfusions. |
| Cytogenetic CR (CRc) | Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from BM. |
| CR with incomplete recovery (CRi) | All CR criteria except for residual neutropenia (< 1.0 X $10^3$/UL ) or thrombocytopenia (< 100 X $10^3$/UL) |
| Partial remission (PR) | All hematologic criteria of CR<br>Decrease of BM blast percentage to 5% -25% OR decrease of pre-treatment BM blast percentage by at least 50%. |
| **Treatment failure** | |
| Resistant disease (RD) | Failure to achieve CR or CRi |
| Relapse | Bone marrow blasts ≥ 5%; or reappearance of blasts in the PB; or development of extramedullary disease. |

**Post- Remission Therapy (Consolidation)**

Once remission is induced, further intensive treatment of patients with AML is essential to prevent relapse. Various types of post-remission strategies have been evaluated including intensive conventional chemotherapy and high-dose therapy followed by autologous or allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-matched related or unrelated donor.[6, 53, 59]

To judge the efficacy of the induction therapy, a bone marrow aspirate and biopsy should be performed 7 to 10 days after completion of induction therapy. In patients who have received standard-dose cytarabine induction and have residual blasts without hypoplasia, additional therapy with standard-dose cytarabine and anthracycline should be considered. For those with signifcant residual blasts or clear-cut induction failure, escalation to high-dose cytarabine with or without an anthracycline is the most common salvage strategy. Other options include an allogeneic HSCT if a matched sibling or alternative donor has been identifed, or participation in a clinical trial. For patients whose clinical condition has deteriorated such that active treatment is no longer appropriate, best supportive care should be continued. If the marrow is hypoplastic (defned as cellularity < 10%–20% and residual blasts < 5%–10%), additional treatment selection may be deferred until marrow recovery, when the remission status can be assessed.[13]

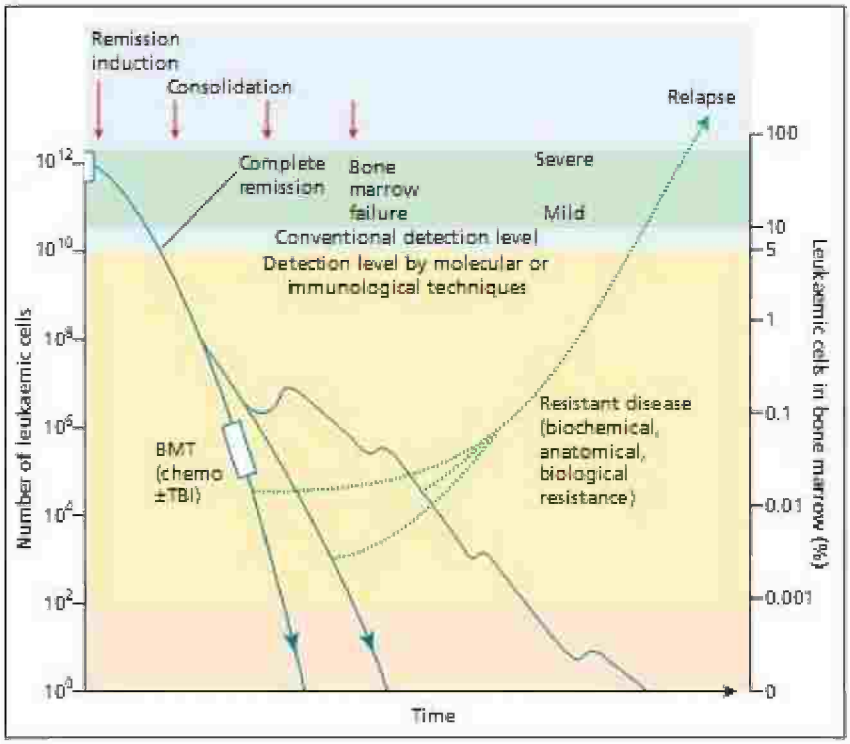



**Figure (11):** Diagnostic representation of treatment strategy.[61]

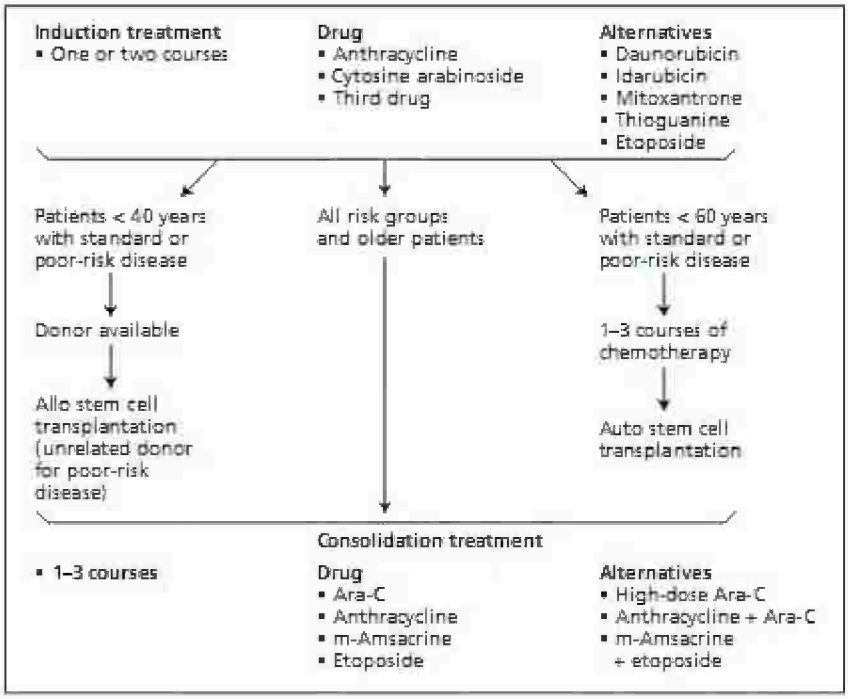



**Figure (12):** Treatment options in AML.[62]

# Telomerases (EC 2.7.7.49)

## Genetic Background

The telomerase reverse transcriptase (TERT) gene is located at human chromosome 5p15.33, and the telomerase RNA component (TERC) gene that encodes telomerase (TR) is at 3q26.3.[63]

Chromosomal gains and gene amplifications involving chromosome arms 5p and 3q are among the most frequent in human tumors. For example, chromosome 5p is often amplified in neuroblastoma, lung cancer, squamous cell carcinoma of the head and neck (SCC-HN), carcinoma of the cervix, medulloblastoma and osteosarcoma.[63]

Amplifications involving 3q have been consistently detected in ovarian carcinoma, carcinoma of the cervix, lung cancer and SCC-HN.[63]

## Structure

### a) Telomeres

Telomeres are ribonucleoprotein complexes at the ends of chromosomes that are essential for chromosome protection and genomic stability. Telomeres consist of tandem repeats of a DNA sequence rich in G bases (TTAGGG in all vertebrates) bound by a six-protein complex known as shelterin. Shelterin encompasses (the Pot1-TPP1 heterodimer, the telomere-binding proteins TRF1 and TRF2, and the interacting factors Rap1 and Tin2).[64]

Telomeric chromatin is also enriched in epigenetic marks that are characteristic of constitutive heterochromatin, such as (histone tri-methylation and DNA hypermethylation) which act as negative regulators of telomere length and telomere recombination.[64]

The length of telomere repeats and the integrity of telomere binding proteins are both important for telomere protection.[64]

Telomere shortening below a certain threshold length and/or alterations in the functionality of the telomere-binding proteins can result in loss of telomeric protection, leading to end-to-end chromosome fusions, cell cycle arrest and/or apoptosis. Telomeres also perform other functions, which include the transcriptional silencing of genes located close to the telomeres (this phenomenon is termed subtelomeric silencing), as well as ensuring correct chromosome segregation during mitosis.[64]

### b) Telomerase

Telomerase is a cellular enzyme capable of compensating progressive telomere attrition through de novo addition of TTAGGG repeats to the chromosome ends. Telomerase encompasses a catalytic subunit with reverse transcriptase activity (TERT), an RNA component (TERC) that acts as a template for DNA synthesis and the protein dyskerin (Dkc1), which binds and stabilizes TERC (Fig 13 &14).[64]

Robust telomerase expression is a feature of pluri-potent stem cells and early stages of embryonic development, although telomerase activity is also present in adult stem cell compartments. Telomerase activity in adult tissues, however, is not sufficient to prevent telomere shortening associated with ageing.[64]

Mutations in the different components of telomerase (TERE, TERC and Dkc1), as well as in some shelterins (Tin2), have been linked to rare human genetic diseases, such as dyskeratosis congenita, aplastic anaemia and idiopathic pulmonary fibrosis. These diseases are associated with the presence of short/dysfunctional telomeres and they all exhibit a characteristic failure in the regenerative capacity of tissues (such as the BM) and severe skin hyperpigmentation.[64]

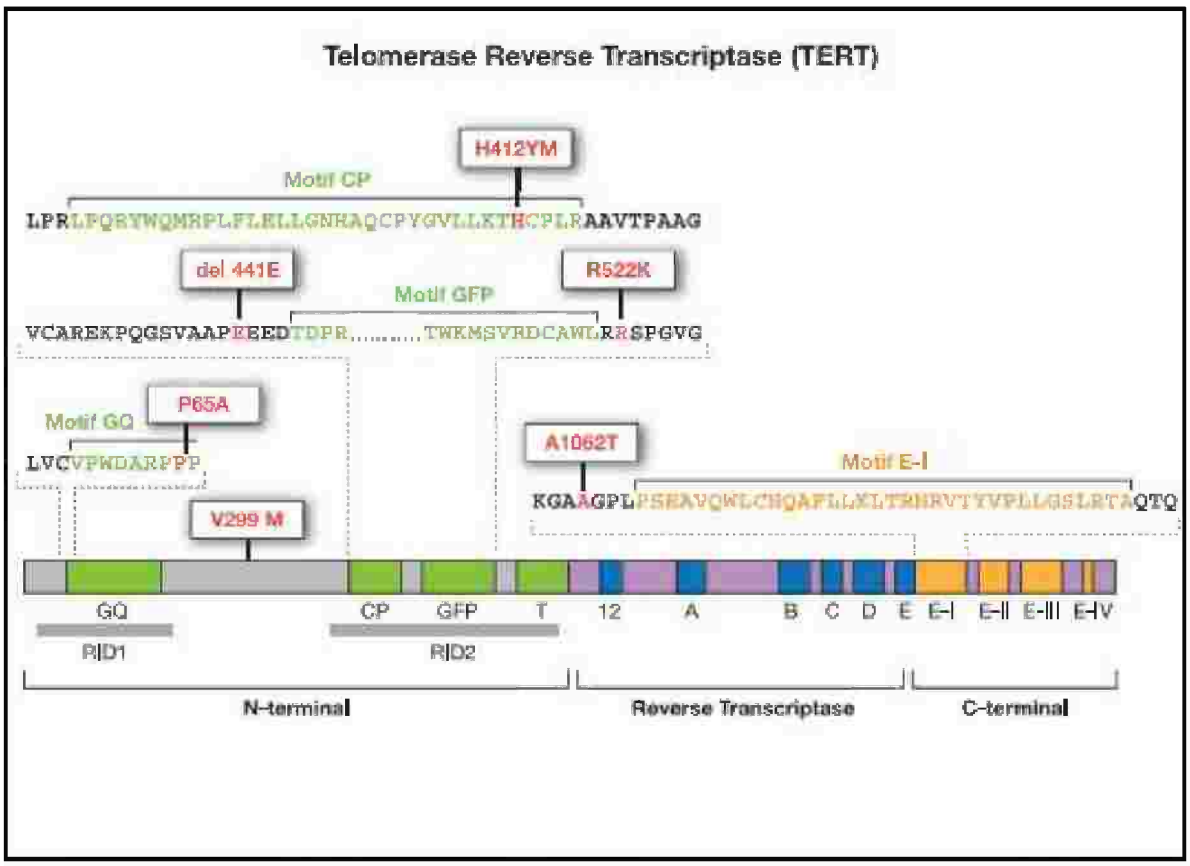



**Figure (13):** TERT mutations in AML.
Schematic domain structure of TERT, indicating 3 major regions: N-terminal, reverse transcriptase motifs, and C-terminal. RID denotes RNA interaction domain and T telomerase-specific motif. Mutation codon locations and amino acid substitutions caused by mutations are shown.
Abbreviations for amino acid residues: A , alanine; E, glutamic acid; H, histidine; K, lysine; M, methionine; R, arginine; T, threonine; V, valine.[65]

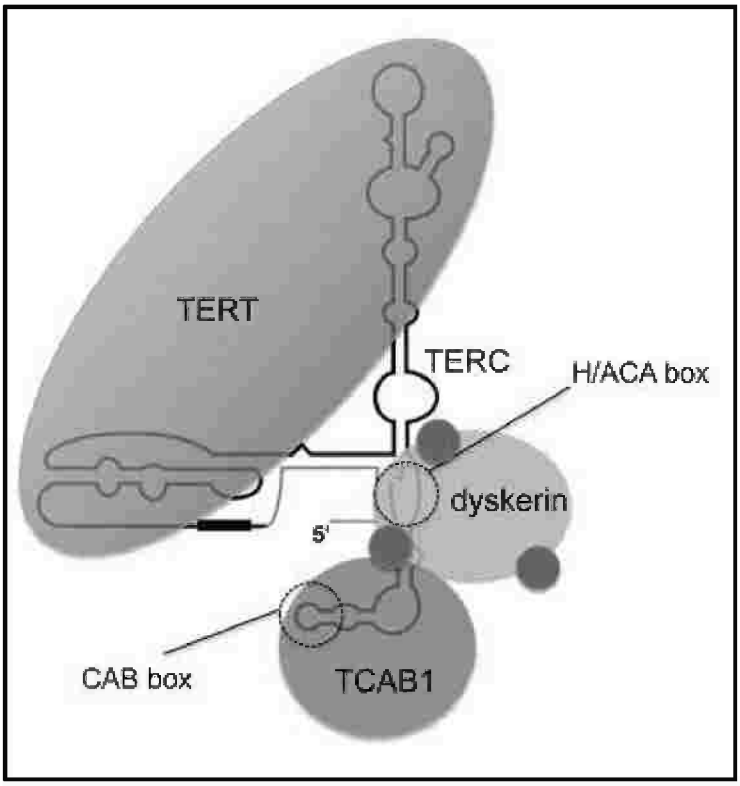



**Figure (14):** Schematic representation of Telomerase enzyme showing its main components.
Telomerase is a large multisubunit RNP. The minimal catalytic core is composed of TERT, the telomerase reverse transcriptase, and TERC, the telomerase RNA, which acts as the template for telomere addition. The 5' end of TERC contains the template region and 3' end of TERC contains two sequences that act as binding sites for additional telomerase protein factors. The H/ACA box represents the binding site for dyskerin, a protein critical for telomerase assembly and for stability of TERC. Dyskerin has three small associated proteins (NHP2, NOP10 and GAR1). TCAB1 is a WD40 repeat protein that recognizes the CAB box in TERC. TCAB1 interacts with dyskerin and is crucial for facilitating telomerase trafficking to Cajal bodies and for telomere maintenance.[66]

## Function (Telomere Biology)

Telomeres are essential for genomic stability (Fig 15), as they protect chromosomes from recombination, end-to-end fusion, and recognition as damaged DNA (double-stranded breaks). With each cell division, telomeric repeats are lost because DNA polymerases are unable to fully duplicate the very ends of linear chromosomes (the end replication problem) and also because of C-strand degradation. Loss of repeats eventually produces critically short dysfunctional telomeres unable to adequately cap chromosome ends, resulting in limited proliferative capacity, cell senescence, apoptosis, and genomic instability.[65]

Telomerase has fundamental roles in bypassing cellular aging and in cancer progression by maintaining telomere homeostasis and integrity. There are also novel biochemical properties of telomerase in several essential cell signaling pathways without apparent involvement of its well established function in telomere maintenance (the extracurricular activities of telomerase) like apoptosis, DNA repair, stem cell function, and in the regulation of gene expression.[67]

Most human somatic cells have undetectable telomerase activity due to transcriptional repression of the catalytic subunit hTERT during early embryonic development. Consequently human somatic cells lose telomeric DNA in cell culture by 50-200 base pairs after each round of replication. Thus, normal somatic cells without telomerase activity can only proliferate a limited number of times in culture. Telomere shortening also occurs with increased age in vivo. It is believed that when one or more telomeres become critically shortened, the protective function of the telomere is compromised. The dysfunctional telomere(s) is recognized as damaged DNA, which triggers a permanent growth arrest known as replicative senescence. The senescence phenotype can also be induced by insults including oxidative stress, overexpression of certain oncogenes, and DNA damage signals. This type of senescence was originally termed premature senescence, even though morphologically both premature and replicative senescent cells appear similar.(67)

Telomerase is necessary for the long-term proliferation potential of human stem cells and cancer cells, and for normal tissue renewal. Ectopic expression of telomerase in normal human cells leads to extension of life-span or immortalization of many cell types. Inhibition of telomerase in telomerase positive cancer cells can lead to the induction of cell death, also mutations in either hTR or TERT are associated with many premature aging phenotypes leading to human diseases.(67)

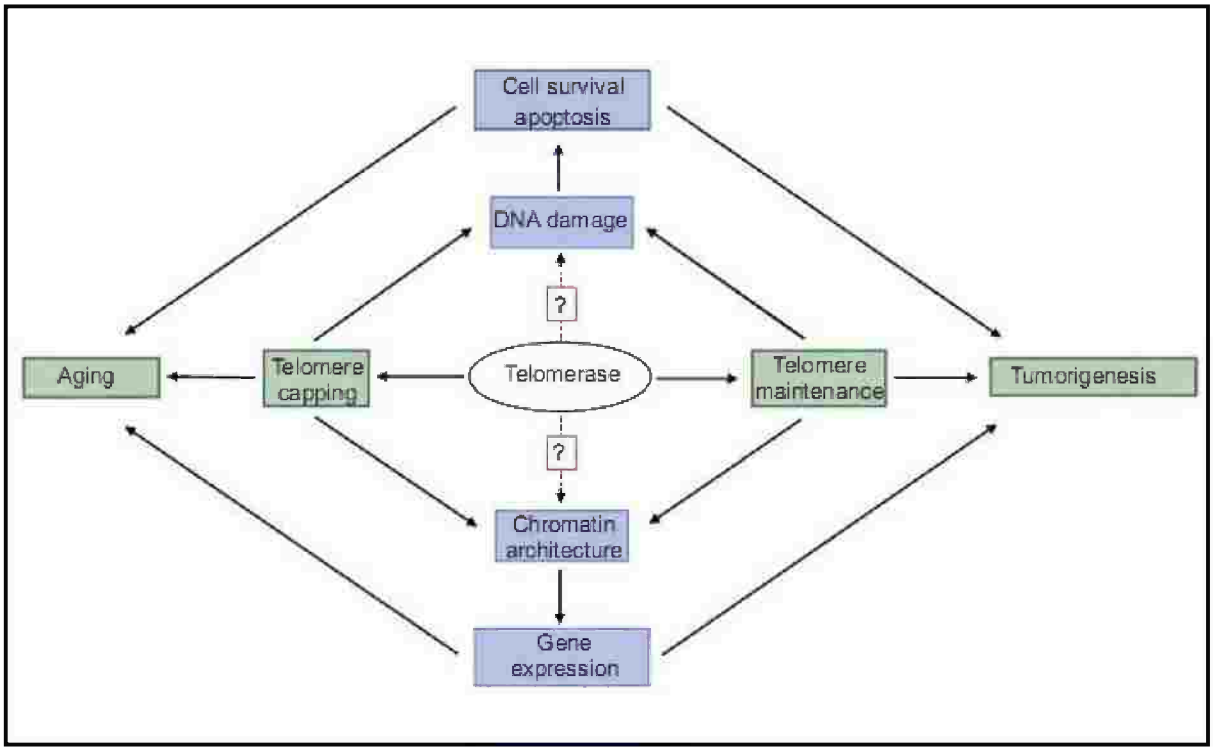



**Figure (15):** Potential pathways of telomerase actions linked to aging and cancer. Telomerase maintains telomere integrity by elongating telomere length and capping telomeres. Additionally, telomerase may be involved directly or indirectly in DNA damage response, in regulation of chromatin architecture and in regulation of gene expression, collectively contributing to aging and cancer.(67)

## Telomerase Level

The RNA component hTERC is ubiquitously expressed in all tissues, so expression of hTERT is believed to be the limiting factor for telomerase activity. Indeed, a close correlation between the expression level of hTERT mRNA and telomerase activity has been described in human cells.[68]

Telomerase activity and particularly telomere length differs between embryogenic and fetal stem cells as well as with ontogenetic age of the hematopoietic stem cell source from fetal liver to cord blood to adult BM. Thus, very high levels of telomerase have been detected in fast proliferating embryogenic stem cells while lower levels of telomerase activity have been found in most adult stem cells of neuronal, dermal, intestinal crypt, mammary epithelial, pancreatic, adrenal cortical, renal and mesenchymal origin. In addition increased telomerase activity has been observed in rapidly proliferating cells such as haematopoietic progenitor cells, activated T cells, germinal center B lymphocytes, as well as in the majority of human cancers.[68]

## Role in Cancer

Numerous types of cancer, including AML, use telomerase activity to maintain telomeres and prevent replicative senescence or apoptosis (Fig 16).[69]

In most cancers, telomerase is expressed at levels that are substantially higher than in normal cells. A known consequence of telomerase up regulation (which is considered to play a critical role in oncogenesis) is maintenance of telomere length, and thus evasion by cancer cells of apoptosis that are associated with the steady decrease in telomere length in normal cells. Also telomerase up-regulation confers other advantages on cancer cells independent of its enzymatic activity, this frequently results from increased copy number of the genes encoding telomerase components; The TERT gene is located at human chromosome band 5p15.33, and the telomerase RNA component (TERC) gene that encodes TR is located at 3q26.3.[63]

The increased telomerase activity found in cancers prevents telomere shortening, and allows cancer cells to escape apoptosis.[63]

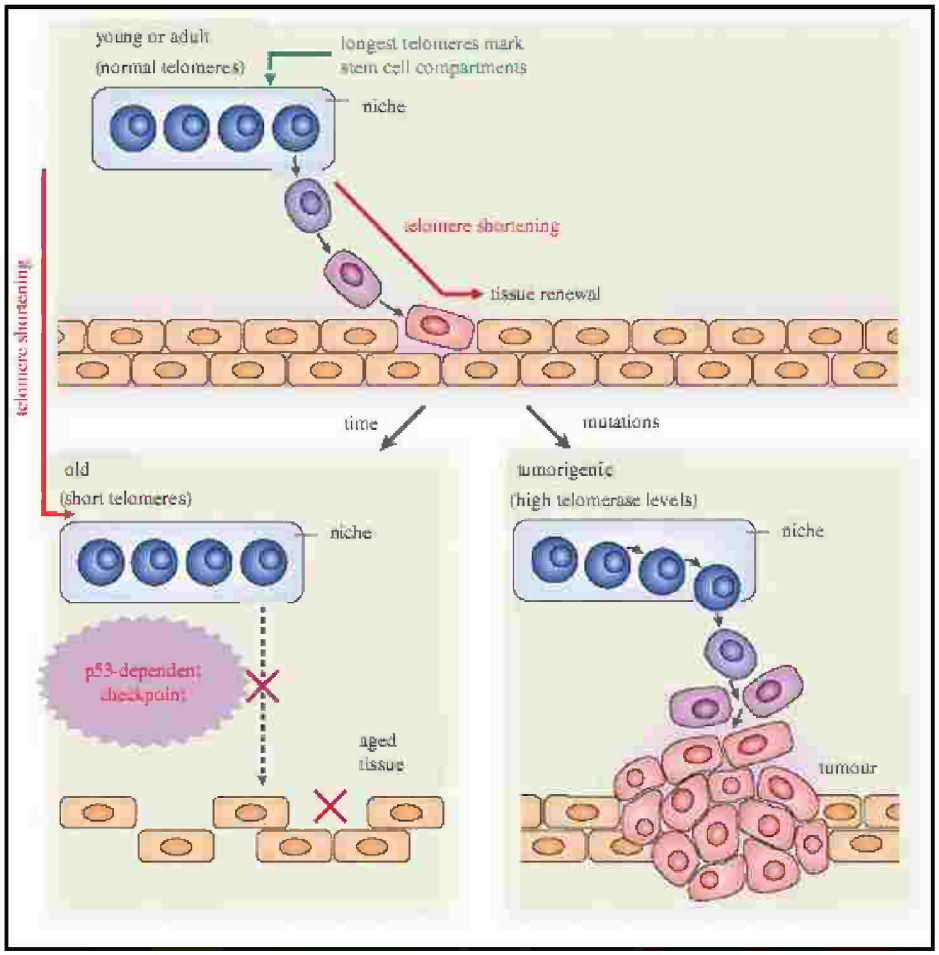



**Figure (16):** A stem cell-based model for the role of telomeres in cancer and ageing. The longest telomeres mark the stem cell compartments (niches). In young or adult organisms, stem cells (blue rounded cells) repopulate tissues as needed: they exit from the niche, proliferate and differentiate (square orange cells). During this process, stem cells undergo telomere shortening, which is partially counterbalanced by the action of telomerase. In old organisms, stem cell telomeres are too short. Critically short telomeres are recognized as DNA damage, activating a p53-mediated DNA damage signaling response that impairs stem cell mobilization and, as a consequence, the tissue regeneration is suboptimal leading ultimately to organ failure. A decreased stem cell mobilization reduces the probability of accumulating abnormal cells in tissues, providing a mechanism for cancer protection. If the stem cells express aberrantly high levels of telomerase (by acquisition of tumorigenic, telomerase-reactivating mutations), stem cell mobilization is more efficient than normal. Under these higher mobilization conditions, tissue fitness would be maintained for a longer time, increasing lifespan and also the probabilities of initiating a tumor.[64]

**Telomeres in CML**

Chronic myeloid leukaemia (CML) serves as a model disease to study telomere biology in clonal MPNs. Most probably due to an increased turn over of the BCR-ABL positive haematopoietic compartment, myeloid cells from patients with CML show accelerated telomere shortening. In CML, telomere shortening correlates with disease stage, duration of chronic phase (CP), prognosis and the response to disease-modifying

therapeutics such as the tyrosine kinase inhibitor, Imatinib. In addition, telomerase activity is already increased in CP CML and further unregulated with disease progression to accelerated phase (AP) and blast crisis (BC).[68]

Furthermore, a correlation of telomerase activity with increased genetic instability as well as a shorter survival of the patients has been reported, accelerated telomere shortening was found to be associated with poor prognosis, It was speculated that increased telomere length was a reflection of an 'earlier' stage of CML CP disease which translates into better response rates.[68]

**Telomerase in AML**

It was suggested that the up-regulation of telomerase activity and the expression of telomerase reverse transcriptase is correlated closely with the occurrence and relapse of AML, so telomerase activity and the expression of telomerase reverse transcriptase may be used to estimate the curative effect and predict relapse of AML. Moreover, the up-regulation of telomerase activity is correlated with the expression of telomerase reverse transcriptase significantly.[70]

Here, we want to discuss the role of telomerase genes amplification in the development and prognosis of AML.

## FISH

FISH is a method by which specific DNA sequences are labeled with a fluorescent tag and applied to metaphase chromosomes or interphase nuclei so that the DNA sequences or probes hybridize to their corresponding sequences within the target cell. The fluorescent tag indicates the presence and position of specific sequences within the cell. FISH may be performed on cytogenetic preparations, tissue imprints, bone marrow smears, or paraffin-embedded sections. FISH is a valuable complementary test to conventional cytogenetics. In cases of poor chromosome morphology, it is capable of accurately detecting subtle abnormalities that are of prognostic importance, such as the inversion 16 in acute myelomonocytic leukemia with abnormal eosinophils, and of detecting cryptic rearrangements and deletions such as the deletion that occurs at the BCR /ABL breakpoint in a proportion of CML cases.[71]

To allow hybridization, target and probe are both denatured to single-strand DNA. This is achieved by heat. The incubation time required for hybridization varies from a few hours to overnight. The specificity of hybridization to target DNA can be controlled by variations of temperature, pH, formamide, and salt concentration in the hybridization buffer. Despite a number of mismatched bases along the strands of DNA, stable duplexes can form under certain hybridization conditions, leading to cross-hybridization or "background." Under conditions of high stringency, only probes with high homology to the target sequence will form stable hybrids, resulting in low or no background hybridization. However, low-stringency conditions (reactions carried out at low temperature, high salt, or low formamide concentrations) may result in high background or nonspecific probe binding. Once the excess probe has been removed in a series of post-hybridization washes, the fluorescent signals are detected via a fluorescence microscope. Scoring of signals and interpretation of results varies according to the probes used and the initial indication for FISH studies.[71]