INTRODUCTION

Acute myeloid leukemia (AML) is a malignant clonal disorder of immature cells in the haemopoietic hierarchical 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. (1)

The more carefully AML is studied, the clearer it becomes that there is considerable heterogeneity between cases with respect to morphology, immunological phenotype, associated cytogenetic, molecular abnormalities and more recently, patterns of gene expression. This is reflected in the substantially different responses to treatment. (1)

Numerous chromosomal aberrations have been documented in acute leukaemias. The underlying mechanisms that drive genomic instability and create the diversity on which clonal selection can operate have not been fully established. However, various lines of evidence have emerged that are consistent with telomere erosion and dysfunction as one possible mechanism driving genomic instability in AML. (2)

Whilst 55% of AML patients exhibit at least one cytogenetically detectable lesion at diagnosis, the remaining 45% do not. It was apparent that 50% of those cases that exhibit normal karyotypes show cryptic sub-microscopic losses or duplications of sub-telomeric DNA encompassing up to 600 kb . (3)

Subtelomeric deletion can arise as a consequence of either a subtelomeric double-stranded DNA break, as these regions are more sensitive to breaks, or the resection of short dysfunctional telomeres. The sub-telomeric break can then be 'healed' by the addition of TTAGGG repeats mediated by telomerase, creating a new telomere and thus stabilizing the sub-telomeric deletion. (4-6)

Telomeres are specialized nucleoprotein structures at the ends of chromosomes; their function is to protect chromosomes from DNA breakage and to prevent chromosome fusion. Without new synthesis, telomeres undergo progressive shortening with each cell division, leading to replicative senescence of cells. Shortening of telomeres can result in telomere end fusions and increase chromosomal instability which is a key intiating event in numerous cancers (7)

Telomerase is an enzyme that extends telomeric repeats on the ends of chromosomes. Activation of telomerase enzyme is therefore required for cells to overcome replicative senescence and to be able to divide indefinitely. Telomerase activity is expressed in germ cells and is present at low level in stem cells, but is usually absent in most somatic cells. Conversely, in immortal cancer cells, telomerase is reactivated, and telomeres are not shortened, suggesting that telomere elongation might be an essential step in tumor formation. (8-9)

Recently genes encoding three major components of human telomerase (TA) have been cloned: human telomerase RNA component (hTR), human telomerase reverse transcriptase (hTERT), and telomerase-associated protein 1 (TAP1). TERT is a telomerase

catalytic subunit that is considered as the key component for the control of telomerase activity. [10-11]

Induction of hTERT expression is essential for telomerase activation during cellular immortalization and tumor progression. Several studies found a relationship between levels of hTERT expression, telomerase activity and clinical aggressiveness of a variety of malignancies. [12-15]

Telomere length (TL) is a key determinant of telomere function. Accurate techniques to measure TL in human tissues have provided a greater understanding of the role of telomeres in the progression to malignancy. [16]

Targeting the hTERT catalytic subunit as anticancer therapy is theoretically tumor-specific and might be less toxic due to its specific expression in tumor and highly proliferating cells compared to other normal cells. Various newly discovered agents represent interesting anti-hTERT candidates for clinical drug development. [12]

The present study was carried out to study telomere length and human telomerase reverse transcriptase (hTERT) level in acute myeloid leukemia and to detect if these parameters might be useful in providing insight into the clinical outcome of AML patients.

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REVIEW OF LITERATURE

Epidemiology of AML

Acute myeloid leukaemia (AML) has an incidence of 2 – 3 per 100 000 per annum 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. (1)

AML is the most common type of leukemia in adults, accounts for 90% of all acute leukemias ,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. The incidence of acute leukemias accounts for <3% of all cancers. These diseases constitute the leading cause of death due to cancer in children and persons age <39 years. (17-19)

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. in the years 1975–2003 was approximately 3.4 per 100,000. The American Cancer Society estimates that 11,930 men and women (6350 men and 5580 women) in the U.S. were diagnosed with AML in 2006.(20, 21)

In Egypt, a series of 83,500 newly diagnosed cancer cases was studied by **The National Cancer Institute (NCI)**, during the period between 2002 and 2010. In 75,036 cases of adults, leukemias constituted 7.7 % of newly diagnosed cancer cases in both sexes. 6417 cases of leukemia diagnosed between 2002 and 2010 in all ages. Sex distribution of 2054 AML cases between 2002 and 2010, males were slightly predominant with male: female ratio of 1.15:1.The median age of adult leukemia was at 42.5 years. (22)

Leukemia ranked as the 4th most common cancer in all ages. In males leukemia is the fourth and the 2nd most common cancer in females. Among 8464 newly diagnosed cancer cases of children in the studied series, leukemia was the most common cancer (represented 29.3% of cancer cases), the most common type was acute lymphoblastic leukemia (represented about 69% of leukemic cases). 564 AML cases diagnosed in children in the studied series. (22)

Etiology of AML

The development of AML has been associated with several risk factors, as summarized in Table I. Generally, known risk factors account for only a small number of observed cases. These include age, antecedent hematologic disease, and genetic disorders; as well as exposures to viruses as well as radiation, chemical, or other occupational hazards and previous chemotherapy. (23)

Leukemogenesis is a multistep process that requires the susceptibility of a hematopoietic progenitor cell to inductive agents at multiple stages. The different subtypes of AML may have distinct causal mechanisms, suggesting a functional link between a particular molecular abnormality or mutation and the causal agent. Most cases of AML arise de novo without objectifiable leukemogenic exposure. [23]

Genetic factors:

Among children, genetic disorders and constitutional genetic defects are important risk factors associated with AML. Children with Down syndrome have a 10-fold to 20-fold increased likelihood of developing acute leukemia. Other inherited diseases associated with AML include Klinefelter syndrome, Li-Fraumeni syndrome, Fanconi anemia, and neurofibromatosis. [24]

Acquired genetic abnormalities:

Acquired (''somatic'') clonal chromosomal abnormalities are found in 50% of AML cases with rising incidences in patients with secondary leukemia or older age. Frequently found abnormalities include loss or deletion of chromosome 5, 7, Y, and 9, translocations such as t(8;21)(q22;q22); t(15;17)(q22;q11), trisomy 8 and 21, and other abnormalities involving chromosomes 16, 9, and 11. [23]

Physical and Chemical Factors

A vast variety of environmental and chemical exposures are assumed to be associated with a variably elevated risk of developing AML in adults. Exposure to ionizing radiation is linked to AML. Among survivors of the atomic bomb explosions in Japan, an increased incidence of AML was observed, with a peak at 5 to 7 years after exposure. Also, therapeutic radiation has been found to increase the risk of secondary AML. [23]

Chemotherapeutic agents, such as alkylating agents and topoisomerase II inhibitors, have been reported to increase the incidence of AML. Chronic exposure to certain chemicals clearly shows an increased risk for the development of AML. Benzene is the best studied and most widely used potentially leukemogenic agent. Persons exposed to embalming fluids, ethylene oxides, and herbicides also appear to be at increased risk. Smoking has been discussed to be associated with an increased risk of developing AML (particularly of FAB subtype M2), especially in those persons aged 60–75 years. [25]

Viruses

Viruses, particularly RNA retroviruses, have been found to cause many neoplasms in experimental animals, including leukemia. However, aclear retroviral cause for acute myeloid leukemia in humans has not been identified to date. An association between the exposure to certain viruses and the development of AML has been suggested. Parvovirus B19 therefore could play a role in the pathogenesis of AML. However, simple infection with either an RNA- or DNA-based virus alone has not been demonstrated as a cause of acute myeloid leukemia. [26]

Secondary AML

The true secondary AML has been recommended to refer to patients who have a clinical history of prior myelodysplastic syndrome (MDS), myeloproliferative disorder, or exposure to potentially leukemogenic therapies or agents; it is thus a rather broad category. Greater than 90% of secondary leukemias are of myeloid origin and have a particularly poor outcome, with a lower incidence of patients achieving a complete remission and a shorter duration of survival than for patients with denovo AML. [23]

Treatment-related secondary leukemia was first observed in survivors of successfully treated Hodgkin disease, which extended later to include survivors of ALL and other disease entities such as multiple myeloma. The development of secondary AML peaks in the5 to 10 years after therapy. [23]

The distinct pattern of cytogenetic and genetic abnormalities in secondary or treatment-related AML is quite remarkable. AML secondary to alkylating agents has: deletions or loss of 7q, or monosomy 7 with normal chromosome 5, and deletions or loss of 5q or monosomy 5. For epipodophyllotoxins, balanced translocations to chromosome bands 11q23, primarily in children, have been described. Topoisomerase II inhibitors have been linked to t(8;21), inv(16). Topoisomerase II inhibitors, anthracyclines,and mitoxantrone, as well as radiotherapy, may be associated with therapy-related acute promyelocytic leukemia with t(15;17) and chimeric rearrangements between PML and RARA genes as well as different translocations to chromosome bands . [23, 27]

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Table (I): Selected Risk Factors Associated With Acute Myeloid Leukemia**.** [23]

Genetic disorders:	Down syndrome Klinefelter syndrome Patau syndrome Ataxia telangiectasia Shwachman syndrome Kostman syndrome Neurofibromatosis Fanconi anemia Li-Fraumeni syndrome
Physical and chemical exposures:	Benzene Drugs such as pipobroman Pesticides Cigarette smoking Embalming fluids Herbicides
Radiation exposure:	Non therapeutic, Therapeutic radiation
Chemotherapy:	Alkylating agents Topoisomerase-II inhibitors, Anthracyclines Taxanes

Clinical presentation of AML

Patients with AML present with symptoms resulting from bone marrow failure, symptoms resulting from organ infiltration with leukemic cells, or both. The time course is variable. Some patients, particularly younger ones, present with acute symptoms over a few days to 1-2 weeks. Others have a longer course, with fatigue or other symptoms lasting from weeks to months. A longer course may suggest an antecedent hematologic disorder, such as myelodysplastic syndrome (MDS). [28]

Symptoms of bone marrow failure:

Symptoms of bone marrow failure are related to anemia, neutropenia, and thrombocytopenia. The most common symptom of anemia is fatigue. Patients often retrospectively note a decreased energy level over past weeks. Other symptoms of anemia include dyspnea upon exertion, dizziness, and, in patients with coronary artery disease,

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anginal chest pain. In fact, myocardial infarction may be the first presenting symptom of acute leukemia in an older patient. [28]

Patients with AML often have decreased neutrophil levels despite an increased total white blood cell (WBC) count. Patients generally present with fever, which may occur with or without specific documentation of an infection. Patients with absolute neutrophil counts (ANCs) < 100 cells/µLhave the highest risk of infection. Patients often have a history of upper respiratory infection symptoms that have not improved despite empiric treatment with oral antibiotics. [28]

Patients present with bleeding gums and multiple ecchymoses. Bleeding may be caused by thrombocytopenia, coagulopathy that results from disseminated intravascular coagulopathy (DIC), or both. Potentially life-threatening sites of bleeding include the lungs, gastrointestinal tract, and the central nervous system. [28]

Symptoms of organ infiltration with leukemic cells

Alternatively, symptoms may be the result of organ infiltration with leukemic cells. The most common sites of infiltration include the spleen, liver, gums, and skin. Infiltration occurs most commonly in patients with the monocytic subtypes of AML. Patients with markedly elevated WBC counts (>100,000 cells/µL) can present with symptoms of leukostasis ie, respiratory distress and altered mental status. Leukostasis is a medical emergency that calls for immediate intervention. Patients with a high leukemic cell burden may present with bone pain caused by increased pressure in the bone marrow. [28]

Physical Examination:

Physical signs of anemia, including pallor and a cardiac flow murmur, are frequently present in AML patients. Fever and other signs of infection can occur, including lung findings of pneumonia. Patients with thrombocytopenia usually demonstrate petechiae, particularly on the lower extremities. Areas of dermal bleeding or bruises (ie, ecchymoses) that are large or present in several areas may indicate a coexistent coagulation disorder (eg, DIC). [28]

Signs relating to organ infiltration with leukemic cells include hepatosplenomegaly and, to a lesser degree, lymphadenopathy. Occasionally, patients have skin rashes due to infiltration of the skin with leukemic cells (leukemia cutis). Chloromata are extramedullary deposits of leukemia. Rarely, a bony or soft-tissue chloroma may precede the development of marrow infiltration by AML (granulocytic sarcoma). [28]

AML classification

In the 1970s, AML was subclassified according to the French-American-British (FAB) classification system using morphologic and cytochemical criteria to define eight major AML subtypes (M0-M7) on the basis of greater than or equal to 30% blasts, lineage commitment, and the degree of blast cell differentiation. The FAB system has been largely replaced by the World Health Organization (WHO) classification, which was developed to incorporate epidemiology, clinical features, biology, immunophenotype, and genetics into the diagnostic criteria. [19]

The WHO has identified seven subgroups of AML. AML is now defined as ≥ 20% myeloblasts, monoblasts or promonocytes, erythroblasts, or megakaryoblasts in the peripheral blood or bone marrow, except in patients with the following cytogenetic abnormalities, who are classified as having AML irrespective of blast count: t(8;21)(q22;q22), inv(16)(p13q22), t(16;16)(p13;q22), and t(15;17)(q22;q12). [19]

Immunophenotypic characterization using surface antigens remains important in AML and may include progenitor-associated antigens (eg, human leukocyte antigen-DR [HLA-DR] [except in APL], CD34, CD117) and myeloid antigens (eg, CD13, CD33); complex composite immunophenotypes, including non-lineage-restricted lymphoid markers, also may be seen. Leukaemic blasts may demonstrate an aberrant immunophenotype, which together with molecular characterization of cloned breakpoints or mutations has potential use in monitoring response to treatment. [1]

Table II: The traditional FAB classification of AML [19]

FAB subtype	**Name**	**Adult AML patients (%)**	**Features**
M0	Undifferentiated leukemia	5%–10%	MPO >3%
M1	Myeloblastic without differentiation	15%–20%	MPO ≥3%, <10% maturation beyond blaststage
M2	Myeloblastic with differentiation	25%–30%	MPO ≥3%, > 10% maturation beyond blast stage
M3	Promyelocytic	10%–15%	≥30 blasts □□hypergranular promyelocytes, strongly MPO or Sudan black B positive; microgranular variant (M3v) has inconspicuous granules,15% of APL
M4	Myelomonocytic	10%–20%	□20% monocytes, NSE positive
	; M4eo - Myelomonocytic with eosinophilia	5%	Abnormal marrow eosinophils, associated with inv(16) or t(16;16)
M5	Monoblastic leukemia; M5a - Monoblastic without differentiation; M5b - Monocytic with differentiation	10%–20%	M5a (poorly differentiated, monoblastic) M5b (differentiated, promonocytes, monocytes); strongly NSE positive
M6	Erythroleukemia	5%	Erythroblasts ≥50%, dyserythropoiesis,glycophorin A(+)
M7	Megakaryoblastic leukemia	5%	Associated with marrow fibrosis, CD41 or CD61often positive

Table (III): WHO classification of AML 2008 [29]

Acute myeloid leukaemia (AML) with recurrent genetic abnormalities
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;q11 – 12),*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* *
Acute myeloid leukaemia with myelodysplasia - related changes
Therapy- related myeloid neoplasms
Acute myeloid leukaemia, not otherwise categorized
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukaemia
Acute monoblastic and monocytic leukaemia
Acute erythroid leukaemia
Acute erythroid leukaemia, erythroid/myeloid
Acute pure erythroid leukaemia
Acute megakaryoblastic leukaemia
Acute basophilic leukaemia
Acute panmyelosis with myelofi brosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis
Acute myeloid leukaemia associated with Down syndrome
Blastic plasmacytoid dendritic cell neoplasm
Acute leukaemias of ambiguous lineage
Acute undifferentiated leukaemia
Acute biphenotypic leukaemia

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Diagnosis of acute myeloid leukemia

Workup for AML includes blood tests, bone marrow aspiration and biopsy (the definitive diagnostic tests), analysis of genetic abnormalities, and diagnostic imaging.

Complete blood count:

A complete blood count (CBC) with differential demonstrates anemia and thrombocytopenia to varying degrees. Patients with AML can have high, normal, or low white blood cell (WBC) counts. [30]

Peripheral blood smear:

Review of the peripheral blood smear confirms the findings from the CBC count. Circulating blasts are usually seen. Schistocytes are occasionally seen if DIC is present. [30]

Bone marrow examination:

Bone marrow aspiration can be performed to detect blast count. Historically, according to the French-American-British (FAB) classification, AML was defined by the presence of more than 30% blasts in the bone marrow. In the newer World Health Organization (WHO) classification, AML is defined as the presence of greater than 20% blasts in the marrow. The bone marrow aspirate also allows evaluation of the degree of dysplasia in all cell lines. [30]

Aspiration slides are stained for morphology with either Wright or Giemsa stain. To determine the FAB type of the leukemia, slides are also stained with myeloperoxidase (or Sudan black), terminal deoxynucleotidyl transferase (TdT) (unless performed by another method [eg, flow cytometry), and double esterase. [30]

Bone marrow biopsy:

Bone marrow biopsy is useful for assessing cellularity. Biopsy is most important in patients in whom an aspirate cannot be obtained (dry tap). Bone marrow samples should also be sent for cytogenetics testing and flow cytometry. [30]

Flow Cytometry (Immunophenotyping):

Flow cytometry (immunophenotyping) can be used to help distinguish AML from ALL and further classify the subtype of AML. The immunophenotype correlates with prognosis in some instances. [31]

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Table (IV): Immunophenotyping of AML Cells [31]

Marker	Lineage
CD13	Myeloid
CD33	Myeloid
CD34	Early precursor
HLA-DR	Positive in most AML, negative in APL
CD11b	Mature monocytes
CD14	Monocytes
CD41	Platelet glycoprotein IIb/IIIa complex
CD42a	Platelet glycoprotein IX
CD42b	Platelet glycoprotein Ib
CD61	Platelet glycoprotein IIIa
Glycophorin A	Erythroid
TdT	Usually indicates acute lymphocytic leukemia, however, may be positive in M0 or M1
CD11c	Myeloid
CD117 (c-kit)	Myeloid/stem cell

Cytogenetic Analysis

Cytogenetic studies performed on bone marrow provide important prognostic information. They are useful for confirming a diagnosis of APL, which bears the t(15;17) chromosome abnormality and is treated differently. Fluorescence in situ hybridization (FISH) studies can be used to get a faster overview of cytogenetic abnormalities than traditional cytogenetic studies. FISH does not replace cytogenetics. [30]

Patients with a good prognosis are those with functional inactivation of the core binding factors (CBFs): These cases include patients with t (8; 21) (q22; q22) or inv (16) (p13; q22), 2 of the most frequent recurrent cytogenetic abnormalities in de novo AML in younger patients. Poor-risk patients have a loss of all or part of chromosome 5 or 7, translocations involving 11q23, or abnormalities of chromosome 3. [32]

Chromosomal translocations:

More chromosomal translocations have been reported in leukemia than in any other cancer. The reason for this is unknown. These translocations frequently lead to the expression of a fusion protein. Although some translocations have been found to contribute to the deregulation of an oncogene without changing its protein product. Most of the translocations in a given disease involve a specific gene, but the partner gene may differ. In addition, some of the translocations can be on the same chromosome, manifesting as an

inversion. The list of translocations reported in various types of leukemias is given in Table III. [33]

Core binding factor (CBF) abnormalities

Core binding factors (CBFs) are transcription factors that play a major role in hematopoietic cell development. Three genes (CBFA1, CBFA2, and CBFA3) encode alpha subunits, which bind DNA, and one gene, CBFB, encodes the non-DNA-binding beta subunit. The t(8;21)(q22;q22) translocation fuses CBFA2(also called AML1 and RUNX1) to ETO (also called RUNXIT1and CBFA2T1), and inv(16)(p13q22)/t(16;16)(p13;q22) similarly disrupts CBFB by fusing the N-terminal portion of the CBF beta subunit to the carboxy terminal of the MYH11protein. These abnormalities are associated with good outcomes in AML, but other translocations involving CBFA2 and additional genes have been reported to be associated with poor outcome. For example, t (3; 21) (q26; q22) results in fusion of CBFA2 to EAP, MDS1, or EVI1and is associated with poor outcome. [34]

Retinoic acid receptor alpha (RARA) abnormalities:

Deregulation of the RARA gene is believed to cause maturation arrest of myeloid cells at the promyelocyte state, leading to acute promyelocytic leukemia (APL). The most common fusion partner gene (90–95%) is promyelocytic leukemia gene (PML), located on chromosome 15, which is fused to RARA as a result of t(15;17)(q21;q22). [34]

However, in 5% to 10% of APL cases, the partner gene is not PML. APL cases with t(11;17) (q23;q21), t(5;17)(q35;q21), and (11;17)(q13;q21) have been reported in which the RARA is fused to the PLZF (promyelocytic leukemia zinc finger), NPM (nucleophosmin), and NuMA (nuclear mitotic apparatus) genes, respectively. [35]

It is important to distinguish these cases due to the fact that combination therapy for APL with all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) is successful except in the presence of PLZF/RARA fusion gene. Mutations in FLT3 have also been reported to be more frequent in APL and to be associated with a higher white blood cell count. [36]

Trisomy 8:

AML cases with trisomy 8 are fairly common (5%), either as the sole abnormality or in combination with other abnormalities. Trisomy 8 is considered of intermediate prognosis. However, some studies suggest that trisomy 8 should be included as an unfavorable cytogenetic abnormality. Multiple important genes have been described on chromosome 8, with the c-MYC oncogene as a leading candidate, but the exact molecular abnormalities in p8 are not defined at present. Most likely this abnormality is associated with additional genomic abnormalites in the leukemic cells that are as yet undiscovered. [33]

Monosomy 5 (-5):

It is common in AML (5%), and is frequently associated with -7, del(7q) or del(17p). In contrast, del(5q) is more common (10%) and more frequently present with additional chromosomal abnormalities in AML. This abnormality is more frequent in older patients, and is usually associated with resistance to chemotherapy and short survival. The pathogenic abnormalities resulting from -5 or 5q- are not known, despite significant work

and research in this field. In -5, there are chromosome 5 species integrated in other chromosomes, raising the possibility that most -5 are, in reality, 5q-. [33]

Monosomy 7 and del(7q) abnormalities:

This abnormality is similar to that described for chromosome 5, and is frequently associated with 5q abnormalities. Monosomy 7 has been reported in 50% of AML cases with inv(3) (q21;q26)/t(3;3)(q21;q26), and is frequently seen at high rates in any abnormality involving 3q21.37 This abnormality is associated with resistance to therapy and poor survival and is seen more frequently in older patients. Furthermore, this abnormality is more frequent in therapy-related AML, especially after alkylating therapy or radiotherapy. [33]

Molecular Marrow Evaluation:

Several molecular abnormalities that are not detected with routine cytogenetics have been shown to have prognostic importance in patients with AML. The bone marrow should be evaluated at least for the commercially available tests. Patients with APL should have their marrow evaluated for the *PML/RARa* genetic rearrangement. When possible, the bone marrow should be evaluated for Fms-like tyrosine kinase 3 (*FLT3*), nucleophosmin (*NPM1*) mutations and Mutations in *CEBPA* (CCAAT/enhancer binding protein α). [30]

Gene-expression profiling is a research tool that allows a comprehensive classification of AML based on the expression pattern of thousands of genes. [30]

Molecular pathophysiology of leukemia

Several molecular changes have also been discovered, either by association with the known cytogenetic abnormalities or somewhat by chance. There is a variable level of proof at this stage as to whether the recognized molecular abnormalities are sufficient in themselves to cause leukaemia. [1]

While cytogenetic analysis is an important part of the diagnostic evaluation in all patients, outcome risk has been difficult to define for patients presenting without chromosome aberrations. However, recently several molecular markers have been discovered that allow for the definition of outcome risk, even in patients with cytogenetically normal AML (CN-AML). That group comprises 40–50% of patients with newly diagnosed disease. Thus, the presence or absence of somatically acquired genetic alterations in both CN-AML and other cytogenetic groups is now an important consideration in risk stratification of these patients. [37]

As a result of the identification of these mutations, patients with CN-AML are recognized as a diverse group with distinct clinical outcomes. Indeed, both the WHO and the European Leukemia Net (ELN) classifications include recurrent molecular abnormalities as a complement to cytogenetics. Aside from risk stratification, the identification of these molecular markers has led to new insights into mechanisms of leukemogenesis and the development of novel targeted therapies. [34, 37]

Gene deletions and amplifications: The deletion of tumor suppressor genes is another mechanism for leukemogenesis. Some of the deletions most recently described

involve the loss of microRNAs (miRNAs) that function as suppressors. Amplifications are rare in leukemias, but have been documented. [33]

Changes in expression: Deregulation of gene expression, such as the down regulation of tumor suppressors or the overexpression of oncogenes, is a common abnormality seen in leukemias. Most instances of downregulation are caused by deletion of the gene, or by hypermethylation. However, some deregulation and downregulation can be caused by other mechanisms that regulate the expression of genes, and they themselves can be deregulated. Post-transcriptional regulation is also well documented. [33]

Methylation : Methylation as a mechanism for the regulation of gene expression has been extensively studied in cancer, and every indication suggests that this phenomenon plays a major role in leukemogenesis. This is particularly important in light of the many methylation inhibitors that are currently being used in treating patients with various types of leukemias.[38]

FLT3 mutation is the most common molecular mutation associated with AML has been found in the FLT receptor. FMS - like tyrosine (FLT) - 3 is a member of the platelet – derived growth factor receptor (PDGFR) subfamily of receptor kinases and is most similar to FMS, KIT and the PDGF receptors. Most mutations are present in the juxtamembrane domain of the receptor and comprise internal tandem duplications (ITDs) of variable size that are always in - frame and therefore expressed. Such mutations are found in approximately 25% of younger AML cases. [39]

Mutations in the activation loop, especially at aspartic acid 835 (D835) residues of the second tyrosine kinase domain (TKD), have also been reported. Most studies suggest that FLT3 mutations lead to poor outcome in patients younger than 65. [40]

Mutations are associated with high white cell counts and blast percentage at diagnosis. They are not uniformly distributed across the FAB or cytogenetic subgroups, being rare in FAB M0, M6 and M7 and most common in M3. The incidence is highest in patients with t(15;17), trisomy 8 and normal karyotype and uncommon in other favourable groups and in virtually all poor - risk cytogenetic groups. The presence of a FLT3-ITD mutation at the time of relapse has also been shown to be associated with a lower rate of achieving a second CR and shorter OS [1,40]

Although clinical trials with tyrosine kinase inhibitors as either single agents or in combination with chemotherapy have yet to show an improvement in the risk of relapse or OS for patients with FLT3 mutations, more potent and specific inhibitors of FLT3 are in development that may have greater clinical benefit. Given the adverse prognostic risk associated with the FLT3-ITD mutation, allogeneic SCT has been recommended in these patients if not entered into clinical trials. [41]

NPM1 mutations: NPM1 gene encodes for a protein that functions as a molecular chaperone that shuttles between the nucleus and cytoplasm to regulate processes within the cell, such as transport of preribosomal particles, responses to stress stimuli and DNA repair, and the activity and stability of tumor suppressors such as p53. Mutations within exon 12 of the gene result in abnormal expression and localization of the protein within the cytoplasm. As the most common molecular marker in patients with CN-AML, NPM1

mutations are found in 45–60% of patients and are associated with achievement of CR and an overall favorable outcome, especially in the absence of an FLT3-ITD mutation.[42]

Clinical features of patients with the NPM1 mutation including an association with myelo-monocytic/monocytic leukemia, elevated WBC counts and the presence of extramedullary involvement. The highest remission rate occurred in those with NPM1 mutated/FLT3-ITD negative disease, a group that also had a more favorable OS. Patients who were NPM1 mutated/FLT3-ITD negative did not benefit from transplantation. These results led to a proposal to reclassify patients with this molecular profile from the intermediate to favorable risk category, and indeed, both the WHO and ELN recognize this new classification. [37]

Owing to the strong predictive nature of this marker, similar chemotherapeutic approaches are now recommended for both CN-AML with mutated NPM1 without FLT3-ITD and core binding factor (CBF) AML, reserving allogeneic SCT until the time of relapse. All-trans retinoic acid (ATRA), an important chemotherapeutic agent utilized in the treatment of patients with APL, has previously been shown to improve the over all survival (OS) of older patients with non-APL AML after intensive chemotherapy, an effect now thought to be due to the presence of leukemic cells with mutant NPM1. [43]

CEBPA mutations : CCAAT/enhancer binding protein α (CEBPA) is a member of the family of basic region leucine zipper transcription factors and is required for the control of metabolic processes, such as glucose metabolism, and granulocytic differentiation. CEBPA mRNA gives rise to two separate translation products, the shorter form known as p30 and the longer form known as p42. The trans activating domains are only present in the p42 isoform, while the domains necessary for interaction with other transcription factors are present in both. CEBPA mutations are found in 10–15% of patients with CN-AML and are typically biallelic. [44]

However, the presence of the mutation predicted for improved DFS and OS, independent of other molecular markers (e.g., FLT3, NPM1 etc). In addition, patients with one affected allele could not be distinguished from wild-type cases with regard to outcome. They expressed a gene-expression profile that was different from those with double CEBPA mutations, who had a more favorable outcome. [45]

IDH1/IDH2 mutations: Isocitrate dehydrogenase (IDH), a member of the β-decarboxylating dehydrogenase family of enzymes, catalyzes the oxidative decarboxylation of 2,3-isocitrate to yield 2-oxoglutarate and carbon dioxide in the Krebs cycle. Mutations within IDH1 and IDH2 encode for an isoform of the protein with a loss of function that both impairs this reaction and creates a gain of function in the reverse reaction that reduces α-ketoglutarate to an oncogenic molecule, 2-hydroxyglutarate . [46]

DNMT3A mutations : Aberrant DNA methylation resulting in epigenetic silencing of structurally normal genes relevant to the regulation of cellular differentiation, proliferation and survival has been described in AML and is considered to play a role in disease pathogenesis. Methylation of the cytosine residue of CpG dinucleotides is catalyzed by one of three isoforms of the enzyme DNA methyltransferase: DNMT1, DNMT3A or DNMT3B. All of which are over expressed in malignant blasts as compared with normal bone marrow cells. [47, 48]

RUNX1 mutations: Chromosomal translocations involving the runt-related transcription factor 1 (RUNX1) gene are well documented and have been observed in AML. As a transcription factor, RUNX1 is involved in the regulation of normal hematopoietic differentiation. Chromosomal translocations or mutations within the gene result in deregulation of its function. [49] In the largest cohort of CN-AML patients treated with cytarabine- and anthracycline-based chemotherapy, Mendler et al. evaluated 392 patients and identified RUNX1 mutations (RUNX1-mut) in 12.5% of patients (8% aged <60 years and 16% aged >60 years). [50]

WT1 mutations: The Wilms' Tumor 1 (WT1) gene, located at chromosomal band 11p13, encodes for a transcriptional regulator with tissue-specific expression and key roles in cellular growth and development. Structurally, the N-terminal domain is composed of proline-glutaminerich sequences necessary for both RNA and protein interactions, while the C-terminal domain is composed of four zinc fingers, which bind directly to DNA sequences to enhance or repress target gene expression. [51]

While not completely understood, the WT1 protein is able to act as both a tumor suppressor and an oncogene. This dual functionality may be due to specific protein–protein interactions, post-translational modifications to the molecule or differential activity of the four isoforms of the protein. [52, 53]

In AML, WT1 mutations occur in 7–12% of patients with CN-AML and their impact on outcome is controversial. A large series of more than 600 patients with CN-AML reported that while WT1 mutations were significantly associated with the presence of FLT3-ITD and CEBPA mutations, there were no differences in rates of CR, refractory disease or OS between patients with mutated or unmutated WT1 disease. However, patients with both a WT1 mutation and FLT3-ITD mutation did have a lower rate of CR and an increased rate of refractory diseases. [54]

MLL mutations: partial tandem duplications (PTDs) of the mixed lineage leukemia gene, MLL (MLL-PTDs), were the first molecular marker described in patients with CN-AML. While MLL is known to be involved in recurrent translocations in both AML and acute lymphoblastic leukemia, MLL-PTD results from a duplication of a genomic region containing either MLL exons 5 through 11 or 5 through 12, which is then inserted into intron 4 of the full-length MLL gene. [55]

The initial description of 98 patients with CN-AML (aged 18–84) reported an aggregate frequency of the mutation of 11% and a shorter duration of CR as compared to those without MLL-PTD rearrangements. Subsequent studies have confirmed the frequency of the mutation in CN-AML and trisomy 13, as well as the short remission duration. [56]

Interestingly, the MLL-PTD protein retains its normal function and is able to contribute to DNA hypermethylation and epigenetic silencing of tumor suppressor genes. In vitro, this can be reversed following hypomethylating and histone deacetylase inhibitor treatment. A clinical trial with this combination of therapeutic agents is ongoing in patients with elapsed or refractory AML.[57]

Other gene mutations : Tet oncogene family member 2 (TET2) gene mutations are known to occur in myeloid malignancies including myelodysplastic syndromes and

myeloproliferative neoplasms and were first reported to have an adverse impact on patients with ELN favorable-risk AML (mutated NPM1 without FLT3-ITD or who have mutated CEBPA). [58-60] Metzeler et al. reported an analysis of 427 patients with previously untreated CN-AML who received cytarabine/daunorubicin therapy and noted an aggregate frequency of the mutation of 23%, with an association with older age and higher pretreatment WBC count as compared with wild-type TET2. [60]

Most interesting was the impact of the presence of this mutation on patients with favorable risk CN-AML. Among this group of patients, those with TET2 mutations had a lower CR rate and a shorter EFS and OS than those with wild-type TET2. While there was no impact of TET2 mutations in the ELN intermediate-I-risk group (defined as CEBPA-wt and FLT3-ITD and/or NPM1 wt). More recently, Gaidzik and colleagues reported a lack of impact of TET2 mutations in patients with CN-AML. In fact there was no difference in the cumulative incidence of relapse, relapse-free survival or OS overall, or those with ELN favorable or intermediate-I-risk disease [61]

Similar to TET2, mutations in the additional sex combs like-1 (ASXL1) gene have also been identified in patients with AML, myelodysplastic syndromes and myeloproliferative neoplasms. In a cohort of more than 400 patients with molecularly well characterized CN-AML who received cytarabine- and daunorubicin-based induction chemotherapy, the impact of ASXL1 mutations on outcome and their association with the disease features were analyzed. [62-65]

In addition, among those patients in this favorable-risk group who did achieve a CR, all ASXL1-mut patients relapsed within 13 months and died within 18 months from diagnosis, where 27% of ASXL1-wt remained disease free at 3 years and had an OS of 34%. There were no significant differences in CR, DFS or OS in ELN intermediate-I genetic risk patients. Therefore, similar to TET2 mutations, ASXL1 mutations aid in the identification of a high-risk subgroup of older CN-AML patients and may have a greater impact on prognosis than TET2 mutations. Indeed, in this cohort of patients with ELN favorable CN-AML, those with ASXL1 mutations had a significantly worse OS as compared with ASXL1-wt/TET2- wt, regardless of TET2 status. [66, 67]

Other gene mutations such as TP53 have been identified in patients with AML, albeit in a lesser frequency than other mutations, which has made it difficult to discern their impact on risk stratification of patients with AML. [66, 67]

miRNA: miRNAs are noncoding RNAs of 19–25 nucleotides that effect protein expression by modulating post-transcriptional activity of mRNA . Deregulation of miRNAs contributes to disease pathogenesis in AML by interfering with the expression of proteins necessary for normal differentiation, proliferation and apoptosis of hematopoietic cells. miRNA-expression profiling is able to distinguish between myeloid and lymphoid leukemia, as well as distinct cytogenetic subtypes of AML based on upregulation or downregulation of specific miRNAs. [68, 69]

In patients with CN-AML, distinct miRNA-expression profiles have been correlated with certain molecular markers. Becker, et al described the gene-expression signature of a series of older patients with CN-AML and NPM1 mutations that featured upregulation of homeobox genes accompanied by higher expression levels of miR-10a, miR-10b, miR-196a and miR-196b. Upregulation of genes involved in erythroid differentiation is a

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distinct feature of the gene-expression signature seen in patients with CN-AML and CEBPA mutations, and miRNA involved in erythroid lineage differentiation. Members of the miR-181 family, are also upregulated in this group suggesting a functional relationship between miRNA and gene expression. [45]

Patients with FLT3-ITD-mutated AML also show aberrant miRNA expression with a two- to threefold increase in miR-155, as well as miR-10a and miR-10b. The discovery of aberrant miRNA expression in AML has led to further insight into mechanisms of disease pathogenesis but has also provided additional prognostic information regarding OS and risk of relapse. For example, increased expression of miR-181a and miR-181b in high-risk CN-AML (NPM1 wild type/FLT3-ITD mutated) is associated with a lower risk of relapse or failing to achieve a CR, which contrasts with the overall poor outcome of this group. Across all cytogenetic subgroups, overexpression of miR-20a, miR25, miR-191, miR-199a and miR-199b adversely affect OS. [70, 71]

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Table V: Examples of genes involved in the cytogenetic abnormalities found in AML[1]

Cytogenetic data	Names of genes	Protein	FAB type
inv(3)(q21;q26)	Ribophorin 1 (*RPN1*) ecotropic viral integration site 1	RER transmembrane glycoprotein, Multiple zinc fi ngers	MDS, M0, M1, M2, M4, M5, M6, M7
t(3;3)(q21;q26)	Ribophorin 1 (*RPN1*) ecotropic viral integration site 1	RER transmembrane glycoprotein, Multiple zinc fi ngers	MDS, M1, M2, M4, M6
t(1;11)(p32;q2)	*AF1P* (1p32) *ALL1* (11q23)	Murine eps 15 homologue *Drosophilia* trithorax homologue	M0, M5
t(1;11)(q21;q2)	*AF1Q* (1q21) *ALL1* (11q23)	No homology to any known protein *Drosophilia* trithorax homologue	M4
t(3;21)(q26;q2)	*EVI1* (3q26) *AML1* (21q22)	Multiple zinc fingers CBF *Drosophilia* runt homologue	MDS
t(3;21)(q26;q2)	*EAP* (3q26) *AML1* (21q22)	Ribosomal protein L22 *Drosophila* runt homologue	MDS
t(6;9)(p23;q34)	*DEK* (6p23) *CAN* (9q34)	Nuclear protein Nucleoporin	MDS, M1, M2, M4
t(6;11)(q27;q2)	*AF6* (6q27) *ALL1* (11q23)	GLGF motif *Drosophilia* trithorax homologue	M4, M5
t(7;11)(p15;p1)	*HOXA9* (7p15) *NUP98* (11p15)	Class I homeobox Nucleoporin	MDS, M2, M4
t(8;21)(q22;q2)	*ETO* (8q22) *AML1* (21q22)	Zinc finger CBF *Drosophilia* runt homologue	MDS, M2
t(9;11)(p22;q2)	*AF9* (9p22) *ALL1* (11q23)	Nuclear protein, ENL homology *Drosophilia* trithorax homologue	M4, M5

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Table V: Examples of genes involved in the cytogenetic abnormalities found in AML Con.[1]

Cytogenetic data	Names of genes	Protein	FAB type
t(10;11)(p12;q23)	*AF10* (p12) *ALL1* (11q23)	Leucine zipper; zinc finger *Drosophila* trithorax homologue	M4, M5
+11	*ALL1* (11q23)	*Drosophila* trithorax homologue	M1, M2
t(11;17)(q23;q)	*ALL1* (11q23) *AF17* (17q21)	*Drosophila* trithorax homologue Leucine zipper; zinc finger	M5
t(11;19)(q23;p13.1)	*ALL1* (11q23) *ELL* (19p13.1)	*Drosophila* trithorax homologue Transcription enhancer	M4, M5
t(11;19)(q23;p13.3)	*ALL1* (11q23) *ENL* (19p13.3)	*Drosophila* trithorax homologue Transcription factor	M4, M5
t(12;22)(p13;q11)	*TEL* (12p13) *MN1* (22q11)	ETS - related transcription factor Nuclear protein	MDS, M1, M4, M7
t(15;17)(q22;q11 – 12)	*PML* (15q21) *RARA* (17q21)	Zinc finger Retinoic acid receptor	M3
inv(16)(p13;q22)	*MYH11* (16p13) *CBFB* (16q22)	Smooth muscle myosin heavy chain Heterodimerizes with AML1	M4Eo
t(16;16)(p13;q)	*MYH11* (16p13) *CBFB* (16q22)	Smooth muscle myosin heavy chain Heterodimerizes with AML1	M4Eo
t(16;21)(p11;q)	*FUS* (16p11) *ERG* (21q22)	RNA - binding protein M1, M2, ETS - related transcription factor	M4, M5

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Management of acute myeloid leukemia

In light of the age distribution of acute myeloid leukemia (AML) decisions on the approach to treatment are primarily determined by patient age. Unless there are compelling reasons to the contrary, patients up to 60 years will be offered intensive induction and consolidation treatment. (33)

Older population can be offered conventional chemotherapy according to patient-related factors such as age, performance score, and presence of comorbidities, social circumstances and the wishes of the patient. Less frequently disease-related factors, such as the presence of high-risk cytogenetics, might suggest that there is little to be gained from conventional treatment. (33)

Induction chemotherapy: Conventional treatment is a combination of an anthracycline and cytarabine (cytosine arabinoside [Ara-C]). The anthracycline is usually daunorubicin (60-90 mg/m^2), mitoxantrone(12 mg/m^2), or idarubicin (12 mg/m^2). Ara-C (100-200 mg/$m^{2)}$ may be given as a 12-hourly bolus or by continuous infusion over 7 to 10 days. Which anthracycline? In terms of overall benefit there are few consistent data to suggest a best choice. In some studies mitoxantrone or idarubicin tended to have a modest advantage, but are associated with more myelosuppression. In an extensive formal meta-analysis of seven comparative trials the conclusion confirmed this perception. (72)

Many studies suggest that 90 mg/m2 of daunorubicin is superior to 45 mg/m2 suggesting that higher doses may provide benefit. However, the outcome in these reports is matched by several other studies in which conventional doses were used. Three major studies have assessed the value of dose escalation of high- or intermediate-dose Ara-C during induction. The observations were that there was no convincing overall survival benefit. (19)

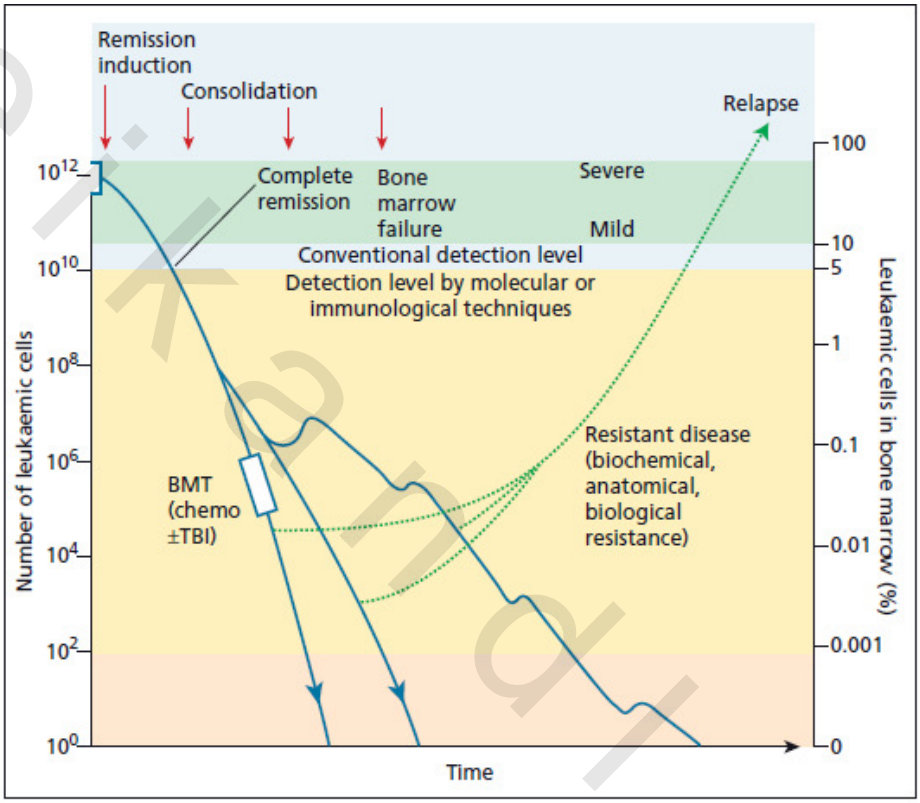
A large study conducted by the Australian Leukemia Study Group suggested that the addition of etoposide as a third drug improved outcome. In a major randomized comparison of daunorubicin plus Ara-C combined with either etoposide or thioguanine as the third drug, no significant differences were observed with respect to remission rate or survival overall or in any patient subgroup. The conclusion for standard practice at the present time is that a combination of anthracycline 3 days and Ara-C by continuous infusion over 7 days, or 12-hourly bolus over 10 days, is standard care. (33)

Results of induction chemotherapy as a general estimate, combination chemotherapy will achieve complete disease remission (CR) in 75–80% of patients under 60 years and is age related. For patients over 60, 45–60%will enter CR. There has been improvement in the remission rate over the last 20 years in all ages. These improvements can probably be accounted for by improved supportive care. (33)

Definition of response to therapy: Complete remission (CR) means an approximate 2 - log reduction in tumour burden. This becomes compatible with a bone marrow that appears normal morphologically and is functionally able to produce normal numbers of circulating cells. The traditional consensus definition of CR is based on these premises: less than 5% blast cells in a cellular marrow durable for at least 28 days with a peripheral neutrophil count of 1.5×10^{9} /L and platelet count above 100×10^{9} /L, and absence of extramedullary disease. (1)

In some cases, these criteria may be met but the morphology is dysplastic. Similarly, some patients meet the marrow criteria but do not achieve full peripheral count regeneration, now called CRi (complete remission with incomplete hematologic recovery) i.e. either the neutrophil or platelet count has failed to reach the level required for CR. This subgroup tends to have a poorer overall survival. [1]

In terms of survival it has been less clear whether CR and CRi were equivalent. In a recent retrospective comparison of two large databases CRi had an inferior overall survival compared with CR by Cheson Criteria. In some studies an early marrow assessment on day 14 has been used as an indicator for immediate additional therapy and poorer outcome. [32]



1

Figure 1 Diagnostic presentation of treatment strategy [1]

Resistance modulation:

A number of biochemical mechanisms can make cells resistant to the frequently used agents such as daunorubicin or etoposide. These include P-glycoprotein (Pgp), which is a product of the MDR1 gene, MRP (multidrug resistance-associated protein), LRP (lung resistance protein), and BCL2. P-glycoprotein over expression is frequently seen in AML, particularly in older patients where more than 70% have this feature. [33]

Retinoic acid (ATRA): The inclusion of ATRA treatment in APL has revolutionized the survival in that disease. APL cells are amenable to the proapoptotic effects of ATRA. Preclinical studies demonstrated that pretreatment of non-APL AML cells with ATRA increased sensitivity to Ara-C, probably by reducing the expression of BCL2. This

provided the rationale for testing the combination of ATRA with chemotherapy in non-APL patients. The first study was initially encouraging but in final analysis showed no benefit, however, has shown a survival benefit in older patients. They also suggested that response was associated with the presence of a mutation of the nucleophosmin 1 (NPM1) gene or the expression of the meningioma1 gene (MN1). These findings have not been confirmed in the large MRC study. [73-75]

Fludarabine-based induction combinations: Fludarabine as a single agent is active in AML but only at doses associated with unacceptable neurotoxicity. The biochemical rationale for combining with Ara-C resulted in several similar schedules of fludarabine/ Ara-C/idarubicin usually with additional G-CSF being developed and tested in the phase II settings. The issue of whether G-CSF or even idarubicin are important contributions has received modest attention. The MRC AMLHR trial compared fludarabine/Ara-C (FA) G-CSF against Ara-C/daunorubicin/etoposide (ADE_G-CSF) in patients with high-risk disease or relapse and found that FA was not superior and the addition of G-CSF did not improve results. [76]

Consolidation chemotherapy:

High dose Ara-C: The Cancer and Leukemia Group B (CALGB) trial 53 established the evidence of an Ara-C dose response. The aim of the trial was to compare three doses in consolidation, 100 mg/m2 versus 400 mg/m2 versus 3.0 g/m2. This established 3 g/m2 as a standard of care for consolidation, which is suitable for patients with favorable cytogenetics and young age. [77]

Stem cell transplantation: Using a donor as a source of stem cells has the additional benefits of ensuring that the new marrow is not a source of disease, but more importantly provides a graft-versus-leukemia (GVL) reaction. There is a growing belief that it is the GVL effect that mediates the major component of the antileukemic mechanism. [33]

The UK MRC AML10 trial evaluated consolidation chemotherapy with or without an additional transplant. There is a consistent picture that transplant reduces the risk of relapse in most risk groups and age strata; this results in a significantly better disease-free survival, but not overall survival. This discrepancy is explained by the fact that there remains an important procedure-related mortality, and that a higher proportion of patients who received chemotherapy alone could be salvaged when they relapsed, compared with those who were transplanted. [78]

It is still accepted practice to offer high-risk patients this treatment approach with the recommendation that patients should be identified early and move to transplant promptly. This is also justified by the poor chance of salvaging poor-risk patients if they relapse. The remaining difficulty remains with patients who are neither good nor poor risk, who comprise 60% of patients in the transplantable age range. None of the studies showed an overall survival benefit in these standard-risk patients. [33]

Cytogenetics is not the only determinant of relapse risk other features such as presenting white count, age, de novo or secondary disease, and marrow response to first treatment course are all readily available factors, each of which is an independent predictor. All or any of these factors may cause concern in patients who are of standard cytogenetic risk. [79]

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The molecular heterogeneity of AML has delineated mutations which have prognostic implications, for example mutations of NPM1 and CEBPα tend to be favorable whereas FLT3, EVI1, and WT1 mutations predict for a higher relapse risk. Setting these additional factors in the context of existing factors and using them to direct therapy is a major challenge which is now beginning.[33]

While defining which patients will have their survival improved by a standard myeloablative transplant is very complicated, it has also been limited in its applicability by the need to find a matched donor and by age. There was no evidence of a survival benefit for patients >35–40 years of age. New transplant techniques have now opened up opportunities for a substantially greater proportion of cases. [78]

The recognition that myeloablative treatment was not a prerequisite for establishing a full chimera has raised the prospect in AML that a reduced intensity conditioned (RIC) allograft could confer the same GVL effect. This is a feasible option for older patients with encouraging survival. This approach has not been proven to be superior to chemotherapy and should therefore be undertaken in the context of a clinical trial. [80]

Transplantation in second remission: Overall transplantation in second remission improves survival from 20% to 40%. The most powerful predictors of outcome if a patient relapses and enters a second remission are age, duration of CR1, and the original cytogenetic risk group. Of those receiving a transplant and using a donor versus no donor assessment, good- and intermediate-risk but not poor risk patients had a survival benefit. Similarly patients with CR1 of <6 months did not benefit.[81, 82]

Azacitidine (Vidaza): it is a pyrimidine nucleoside analog of cytidine. It interferes with nucleic acid metabolism. It exerts antineoplastic effects by DNA hypomethylation and direct cytotoxicity on abnormal hematopoietic bone marrow cells. Hypomethylation may restore normal function to genes critical for cell differentiation and proliferation. Nonproliferative cells are largely insensitive to azacitidine. It is indicated to treat myelodysplastic syndromes (MDSs) and is FDA approved for all MDS subtypes. [83]

Panobinostat given semi-sequentially with azacitidine was a feasible and promising clinical regimen for patients with high-risk MDS or AML. The advantages of this combination over chemotherapy include (1) the low rate of early mortality in elderly unfit patients, (2) the promising response rates in patients with a poor-risk karyotype, including patients with mixed lineage leukemia,JAK2 and K-RAS mutant AML, (3) the potential for clinical benefit, even in patients not achieving a clinical response (median OS 7 months; and (4) the low rate of AML transformation in patients with MDS. The demonstration of increased acetylation using a simple peripheral blood mononuclear cell flow cytometry assay during the first month provides a clinically relevant biomarker for defining those most likely to benefit from the addition of panobinostat to azacitidine-based therapy [83]

Decitabine (Dacogen): The majority of patients with AML are elderly and have a poor prognosis despite induction therapy. Decitabine, a DNA-hypomethylating agent that induces differentiation and apoptosis of leukemic cells, is a well-tolerated alternative to aggressive chemotherapy. It is currently FDA-approved for myelodysplastic syndrome, including patients with 20%–30% bone marrow blasts. [84]

Recent clinical attention has focused on evaluating decitabine as frontline therapy for untreated high-risk elderly AML patients. A large randomized international phase III study comparing decitabine to supportive care and cytarabine in elderly AML patients demonstrated significantly improved complete remission rates, but the survival difference did not reach significance. Due to this, decitabine did not achieve FDA approval for AML, but continues to be used off-label. (84)

Growth factors: granulocyte colony-stimulating factor [G-CSF] or granulocyte–macrophage colony-stimulating factor [GMCSF]) these have been used to curtail the duration of post-induction neutropenia. Some studies have intended to prime leukemic cells by exploiting the fact that they have growth factor receptors which can stimulate cells into cycle and thereby make the cells more sensitive to chemotherapy. (85)

Prognostic factors:

What is poor prognosis for chemotherapy is usually also poor prognosis for transplantation. However, there are emerging situations where relevant new therapeutic possibilities are available, e.g., FLT3 inhibitors for FLT3 mutated patients. (33)

Validated prognostic factors: Age, cytogenetic risk group, response to course 1 of treatment, presenting white count, and de novo, secondary, or treatment related disease are among the most widely recognized, and are used to make treatment decisions. (33)

The subtlety of cytogenetic risk groups and prognosis continues to evolve and enter new classifications (Table IV). In general age is the first discriminant in management. While this is a continuous variable, age becomes important in that there are limitations to the intensity of treatment that can be given to older patients, which would be routine in younger patients. The independence of cytogenetics is well validated. (33)

The intermediate cytogenetic risk group comprises 60% of all patients under 60 years. Once again the transplant trials have not consistently shown a survival benefit in this group although a significant reduction in relapse risk and disease-free survival is apparent. This discrepancy is due to the negative effect of greater treatment-related mortality in transplant recipients, and an increased ability to salvage patients who relapse from chemotherapy. It is primarily in this intermediate-risk group that the new molecular information has emerged – much of which appears to lack prognostic implications – some of which has been prognostically validated. (33)

In older patients prognostic factors are more difficult to find, and less useful, because treatment outcome remains poor. However, the same factors apply, but with less discrimination than in younger patients. In general older patients tend to have a preponderance of unfavorable factors and a higher proportion of cases with a chemotherapy “resistant” phenotype. (33)

Molecular information: Several publications have indicated that 25–30% of patients will have a mutation of the growth factor receptor FLT3. This mutation has little effect on induction treatment outcome, but is highly predictive of patients likely to relapse, particularly in the intermediate cytogenetic risk group. Whether this automatically identifies patients who should be transplanted is not yet clear. The situation is further

complicated by the observation that the relapse risk is not the same for all allelic ratios with high mutant:wild type presenting a higher risk. [86]

Increasing complexity has been introduced by the discovery that the cytoplasmic shuttling protein NPM1 is mutated in a high proportion (50%) of patients with a normal karyotype and consistently appears in 60% of patients with an FLT3 mutation. In general NPM1 predicts for a better response to induction and a reduced risk of relapse, and is therefore a favorable factor when it occurs on its own. It also appears to counteract the negative impact of FLT3. So the poorest outcome occurs in FLT3 mutants who do not have an NPM1 mutation. Patients who have an NPM1 mutation without mutant FLT3 have a survival similar to that of favorable (core binding factor) leukemias. [33]

It also appears that a double mutant of CEBPα confers a favorable outcome while EVI1 and BAALC overexpression have a negative impact. A major challenge is to integrate them into patient management. Some will emerge as therapeutic targets such as FLT3 or cKIT within the core binding factor leukemias. [33]

Most guidance recommends that patients with high-risk cytogenetics should proceed to transplant as soon as possible, but even then the overall survival benefit is not unanimous between all studies when subjected to a donor versus no donor analysis. Opinion generally accepts that survival is not improved by transplant in favorable-risk patients (t(8;21), inv(16)), or intermediate risk with NPM1 mutation. It is now recognized that 20–30% of core binding factor leukemias have a cKIT mutation, and that this confers a higher risk of relapse. [87]

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Table VI: Variation in cytogenetic risk group classification in AML[19]

	MRC	SWOG/ECOG	CALGB	GIMEMA/ AML10	German AMLCG
Favorable	t(15;17) t(8;21) inv(16)/ t(16;16)	t(15;17) t(8;21) (lacking del (9q), complex i.e. >3 unrel abn) inv(16)/t(16;16)/ del (16q)	t(15;17) t(8;21) inv(16)/t(16;16)	t(15;17) t(8;21) inv (16)/ t(16;16)	t(15;17) t(8;21) inv(16)/t(16;16)
Intermediate	Normal Other noncomplex	Normal þ6, þ8, -Y, del (12p)	Normal Other non-complex	Normal -Y	Normal Other non-complex
Adverse	abn(3q) -5/del(5q) -7 complex (>5 unrel abn) (excluding those with favorable changes)	abn(3q), (9q), (11q), (21q), abn(17p) -5/del(5q) -7/del (7q) t(6;9) t(9;22) complex (>3 unrel abn)	inv(3)/t(3;3) -7 t(6;9) t(6;11) t(11;19) þ8 complex (>3 unrel abn) (excluding those with favorable changes)	Other	inv(3)/t(3;) -5/ del(5q) -7/del (7q) abn(11q23) del(12p) abn (17p

Minimal residual disease (MRD)

The emerging molecular knowledge and improved fluorescence activated cell sorting (FACS) technology have provided opportunities to monitor disease response at a level of detection well beyond conventional microscopy. There are several studies which provide clear evidence that patients at higher risk of relapse can be identified 3–6 months before clinical relapse. It is important to be aware that the detection of (MRD) does not always predict relapse. Most information available so far derives a threshold of detection that best correlated with relapse risk. On a retrospective analysis; fewer have been prospectively validated and could be treatment dependent. [88]

The paradigm for this approach is APL where detection at a transcript level of 1_10^4 at the end of treatment predicts a high – almost inevitable – risk of relapse. However, since most cases are PCR negative at this point, more relapses come from the patients who are PCR negative at this time. So although the risk is lower, this group is bigger so a larger number of patients who relapse come from that group. The substitution of real-time quantitative (RQ)-PCR which has value in assessing the quality of the RNA does not add

much if used as a single point assay; however, careful sequential monitoring of bone marrow at 3-monthly intervals is more successful. [88]

The implication of MRD detection is that therapeutic intervention at the time of MRD positivity improves survival, compared to treating at the time of hematologic relapse. This is far from being established because in most cases a therapeutic option has not been validated. The exception to this is APL where intervention with arsenic trioxide is very effective. MRD is clearly an additional prognostic factor, but what additional information it provides beyond those which are currently available requires more careful study.[88]

Novel agents: Several novel treatments are currently under evaluation but are not yet approved.

Antibody-directed chemotherapy: Initial studies using a naked anti-CD33 humanized monoclonal antibody showed some activity in acute promyelocytic leukemia (APL) by converting patients who were in remission, but molecularly positive, to molecular negativity. However, in a randomized trial in relapsed older AML, when it is added or not to conventional chemotherapy there was no improvement in remission rate.[89]

An immunoconjugate as Gemtuzumab ozogamicin (GO; Mylotarg) was developed . It is an IgG3 humanized anti-CD33 monoclonal antibody which is attached by a unique chemical linker to the powerful intercalator, calicheamicin. In this context reinduction rates (CR and CRi) of approximately 1 in 3 were observed. When given at full dose, i.e., 9mg/m2, in patients who have had or will have a stem cell transplant, extra liver toxicity in the form of venoocclusive disease (VOD) – otherwise described as sinusoidal obstructive syndrome – was observed. [90]

In older patients who are fit the combination of daunorubicin/Ara-C is being compared with daunorubicin/clofarabine each with or without GO as induction. In younger patients two doses (3 mg/m2 versus 6mg/m2) are being compared in induction (AML17 trial). In the early unrandomized studies of GO the licenced dose of 9 mg/m2 was used as monotherapy. [90]

Clofarabine is a new-generation purine analog that was engineered to capture the advantageous properties of fludarabine and cladribine with a potential for oral dosing. Preliminary studies by the MD Anderson group established efficacy in relapsed acute leukemia. In phase II evaluation in relapsed AML in a schedule of 40 mg/m2 on day 1–5 over 40% of patients achieved a CR which is encouraging for a single agent. [91]

Its efficacy and future potential for oral administration encouraged its evaluation as a single agent in older patients who would not normally be offered standard chemotherapy predominantly on age criteria. Although separate and with slightly different entry criteria, three unrandomized phase II trials have delivered similar and encouraging results. [92]

The investigators believe that it would have been best supportive care or low-dose Ara-C rather than standard chemotherapy, in which case the results give grounds for optimism, particularly with respect to the response to patients with poor-risk cytogenetics. There is a biochemical rationale for combining clofarabine with Ara-C. One completed randomized study using the unconventional “adaptive” randomization design suggested that clofarabine combined with low-dose Ara-C was superior to clofarabine alone. Several

prospective studies are underway or being established to evaluate this agent in a phase II setting. Although in receipt of regulatory approval in advanced ALL in young patients, it is not yet approved in AML. [93]

Farnesyl transferase inhibition Although only mutated in 12–15% of cases, and not being of prognostic significance, inhibition of RAS is a strategy of interest. Farnesyl transferase is required in the essential process of prenylation of cytoplasmic proteins to enable attachment to the inner aspect of the cell membrane to enable signaling. Target proteins include RhoB, RAC, and transforming growth factor beta (TGF-b) as well as RAS. If leukemic cells are dependent on the relevant pathway activation then farnesyl transferase inhibition may be an effective treatment. [94]

A large phase II study in relapse showed a modest response with a CR rate of 6%. A similarly large study in older unfit patients produced a remission rate of around 15–18%. Of interest, potential durable benefit in patients who achieved only a partial response, not dependent on the presence of a RAS mutation. However, when assessed in older patients randomized against best supportive care no survival benefit was observed and the remission rate was less than 10%. While disappointing, prospective studies in combination with chemotherapy are now in progress. [94]

Cloretazine is a novel alkylator of the sulfonyl hydrazine class which was initially shown to be effective in relapsed disease. In a first-line study in older patients (>60 years) a 41% CR rate following a single infusion of 600 mg/m2 was seen. The responses seen included patients with poor-risk cytogenetics, poor performance score, and aged over 70 years. The survival in responders at 12 months was approximately 30%. This encouraged an ongoing randomized trial in patients in first relapse in combination with Ara-C 1.5 g/m2 versus Ara-C alone. This important trial has still to complete recruitment. [97]

Cloretazine associated with significant myelosuppression and possible pulmonary toxicity that implies that testing of different doses and in combinations is justified in order to assess its potential.[95]

Future approaches:

As single agents these have not lived up to the preclinical promise, but there is considerable momentum to examine them in combination with chemotherapy. This will probably be the pattern of their development. Among such strategies are agents which are antiapoptotic, anti-VEGF (vascular endothelial growth factor), anti-KIT, etc. Epigenetic mechanisms may well be relevant and clinical data from studies in myelodysplastic syndromes have shown efficacy which may be applicable to older patients in particular. This concept is being tested in ongoing randomized trials. [33]

Telomeres

With each cell division, replication begins with the separation of the double-stranded chromosome then new bases are added in the 5′ to 3′ direction. But the extreme end of the chromosome is not replicated and it progressively shortens. This is known as the **end-replication problem**. Fortunately, this problem does not result in the loss of essential genes because chromosomes are capped with long repeats of noncoding DNA bases called **telomeres**. [96]

Telomeres, (from the Greek *telos,* meaning end, and *meros,* a component) are specialized nucleoprotein structures at the ends of chromosomes; formed of over 1000 short base DNA sequences, (5'-TTAGGG-3'), repeated several times and bound to specific telomere-binding proteins. Fig.(2) [97, 98]

After many cycles of cell divisions, most of the telomeric DNA is lost and the telomeres reach a critically short length that can no longer protect the chromosomal ends. As a result, the chromosomes become unstable, fused, or lost. Cells with such defects not only are unable to divide, but also may not survive. Once telomeres have shortened to a certain length, cells with intact cellular checkpoint controls (e.g., p53/Rb) undergo **replicative senescence**; and die as a result of apoptosis. As a result, the function of telomeres is very crucial to maintain chromosome physiology and stability. [96,99]

Human telomeres may vary with age and cell type and in general range from 6 to 12 kb in length in somatic cells. Approximately 50–100 base pairs are lost with each cell cycle. [98] There are, however, two distinctive kinds of cells: germ cells and embryonic cells, that must overcome the problem of telomeres shortening, because the body cannot afford to lose them. They solve this problem by maintaining telomere length. [97]

Telomere length is maintained by a specialized reverse transcriptase called **telomerase**. Telomerase gene was recently mapped to 5p15.33. Three major subunits comprising the human telomerase complex have been identified including a human telomerase RNA component (hTR), human telomerase reverse transcriptase (hTERT), and telomerase-associated protein 1 (TAP1). The RNA component (hTR) serves as a template for telomere synthesis. While (hTERT) is a catalytic subunit that is considered as the key component for the control of telomerase activity. Induction of (hTERT) expression is essential for telomerase activation during cellular immortalization and tumor progression. In addition, several other proteins bind to the telomers, these proteins serve essential functions in regulating telomere length, integrity and function. [96-103]

After birth, most somatic cells switch off the activity of telomerase and hTERT transcription is suppressed and subsequantiallty most human somatic cells have undetectable telomerase activity so that it stop division after an average of 60-70 replicative cycles and enter in **replicative senescence**. However, germline cells, stem cells and progenitor cell exhibit telomerase activity, so that those cells can overcome the **replicative senescence** and have higher abilities to divide. [100]

Telomere associated proteins (TRF1, TRF2, POT1, hRAP1, Ku, MRE11, RAD50,ERCC1, XPF, HUS, tankyrase) are important primarily for the stability and regulation of telomere length. Some participate even in the repair of damaged DNA. Multiple additional proteins bind to TRF1 and TRF2 and thus form complexes that regulate the homeostasis of telomeres (Collins and Mitchell, 2002). [104]

Shelterin is a significant protein complex that consists of six subunits (TRF1, TRF2,POT1, hRAP1, TIN2 and TPP1) and participates in forming a cap structure at the end of chromosomes (de Lange, 2005). A higher-order telomere nucleoprotein complex facilitates dynamic change between the cap and loose structure. It presents two states that must be well regulated. The tightly packed loop structure is the limiting factor for the elongation of telomeres as it prevents the access of telomerase and other proteins participating in the elongation of telomeres. [105]

Activation of telomerase enzyme is therefore required for cells to overcome replicative senescence and to be able to divide indefinitely. Furthermore, telomerase has been implicated to have additional activities beyond telomere maintenance, it may be involved in cell survival, apoptosis, DNA damage response, regulation of chromatin architecture, as well as control of gene expression (including genes involved in cell signaling, cell cycle, metabolism, cell death, differentiation and growth factors.[106,107]

Telomerase and cancer:

Telomerase expression is a hallmark of cancer. Nearly all malignant tumors activate telomerase enzyme, indicating the capacity for unlimited proliferation and thus immortality. [108]

Following mutations that initiate clonal expansion, the pre-malignant cell accumulates other critical mutations such as mutation involving p53 genes that results in inhibition of apoptosis and continued cell division with further shortening of telomeres. This repetitive, clonal expansion leads to the acquisition of other mutations, loss of heterozygosity and the ultimate upregulation or reactivation of telomerase that permits the stabilization of the telomeres and an immortal state [108]

The mechanisms that maintain telomere homeostasis play a prominent but complex role in the development of cancer. [97] Cancer cells may maintain telomerase by one of two pathways, a classic pathway in which the telomere is elongated when hTERT, adds new nucleotides using the template sequences of hTR. [100] An alternative mechanism is used by some tumors in the absence of telomerase activity called, alternative telomere lengthening (ALT). The ALT pathway is more commonly activated in tumor of mesenchymal origin and in tumor having the ALT phenotype. This phenotype is usually associated with poor prognosis.[106]

Telomerase might contribute to cancer development independently of its role in maintaining telomere length. It may have role during multistep oncogenesis and tumor progression. Furthermore, activation of telomerase might stabilize karyotypic changes and stabilize chromosomal structure in cancer cells and this allows further proliferation of the immortal clone with acquiring additional genetic alterations advantageous for more aggressive behavior. [107,109]

Regulation of telomerase activity operates at both telomerase gene and protein levels. Regulation of telomerase gene includes epigenetic, transcriptional and post-transcriptional regulation. Epigenetic regulation depends mainly on methylation status of the hTERT promoter. [110-115]

The transcriptional activity of this gene was found to be up-regulated specifically in cancer cells, and silent in most normal cells. [124] A number of binding sites for transcription factors (activators and repressors) have been identified in the hTERT core promoter. [116-119]

Transcriptional factors include some proteins as c-MYC, HIF-1 (hypoxia-inducible factor 1), P53, RB (retinoblastoma), TGF-B (transforming growth factor B), P17, P27 and survivin. Some hormones like progesterone and oestrogen may also act as transcriptional

factors. [115] Several viruses could regulate telomerase transcription; include hepatitis B virus, papilloma virus and more recently avian leucosis virus. [120-122]

Post transcriptional regulation of hTERT results from the differential splicing of hTERT mRNA. This hTERT can exist in a variety of splice forms which results from complete or partial deletions or insertions of axons or interons. [123,124]

Regulation of telomerase protein includes post-translational regulation, cellular localization in addition to the role of telomeric proteins and RNAs in hTERT level regulation. [122]Post-translational regulation of hTERT protein depends on a variety of kinase and phosphatase inhibitors that act at specific protein residues at hTERT molecule.[125] Subcellular dynamic transport of hTERT protein between the nucleoplasm and the nucleolus may have certain implications in the functional regulations of telomerase activity. [126] Furthermore, some proteins can form complex with telomeric DNA and modulate the activity of telomerase. [127-130]

Clinincal implication of telomerase:

Several methods have been developed for the detection of telomerase activity. The telomeric repeats amplification protocol (TRAP) assay is the most widely used method for monitoring telomerase activity. The TRAP assay is highly sensitive and specific for telomerase activity in tumor samples but it has several practical limitations. One limitation is that it is time consuming, another limitation is that because telomerase contains an RNA component, the activity of which is labile and easily destroyed by "RNAase". [131, 132]

Expression of hTERT mRNA is very closely associated with telomerase activity in human tumors and more recently quantitative measurement of hTERT mRNA using real-time quantitative RT-PCR becames a new, useful tool. However, this approach does not offer any information at the level of the individual cell and so comparison of molecular data with cellular morphology is not attainable. [133,134] Therefore, immunohistochemical assay of hTERT protein, which can both detect and localize cellular telomerase expression in tumors, would be optimal for localization of proliferating cells and correlation with histopathological findings. [135]

Telomerase activity has been proposed as an adjunctive diagnostic tool in many cancers. It is estimated that nearly 50% of bladder cancers are missed on initial cytological survey. However, the specificity of telomerase activity in cancer cells allows for earlier detection of bladder cancers in cytology. The combination of telomerase activity to cytologic examination in breast FNA can potentially augment the FNA screening tool in the early diagnosis of breast cancer. [136]

Telomerase activity has all the desired characteristics to be used as a potential cancer-screening tool. It requires a small amount of tissue, can be done on a variety of tissue types or body fluids requiring minimal invasiveness, has a sensitive assay, and is specific to the malignant state in most instances. [137]

Telomerase activity is also thought to increase in direct proportion to grade of malignancy in a series of cutaneous melanocytic lesions, neuroblastoma, breast carcinoma, menengioma, glioblastoma and colorectal carcinoma. In some tumors, high levels of

telomerase may be related to high stage, poor prognosis, dissemination, and cytogenetic abnormalities. [139-140]

Therapeutic potential of telomerase:

The RNA template is a popular target for inhibition of telomerase activity. In vitro studies have shown that the introduction of the dominant-negative (DN-hTERT) into cancer cells inhibits telomerase and leads to progressive telomere shortening and cell death. [137] Preclinical animal studies have demonstrated that telomerase template antagonists (antisense oligonucleotides) are effective in reducing primary growth in variety of cancers. Furthermore, several studies suggested that telomerase template antagonists can reduce the metastatic spread in animal models. [141-143]

Owing to its expression in almost all cancers, telomerase can thus be considered as a potential molecular target for immunotherapy. Recently, a tumor-associated antigen (TAA) that correlates with hTERT expression has been identified in an HLA (human leukocytic antigen) subset of patients. Cytotoxic T-lymphocytes generated against this antigen cause cytolysis in many tumor lines. Several therapeutic vaccines offer the potential to stimulate the rapid killing of tumor cells by enhancing the activity of telomerase specific cytotoxic T cells. Trials have been initiated in several types of solid tumors and in acute myeloid leukemia, todate no significant toxicity to normal tissues has been seen. [144,145]

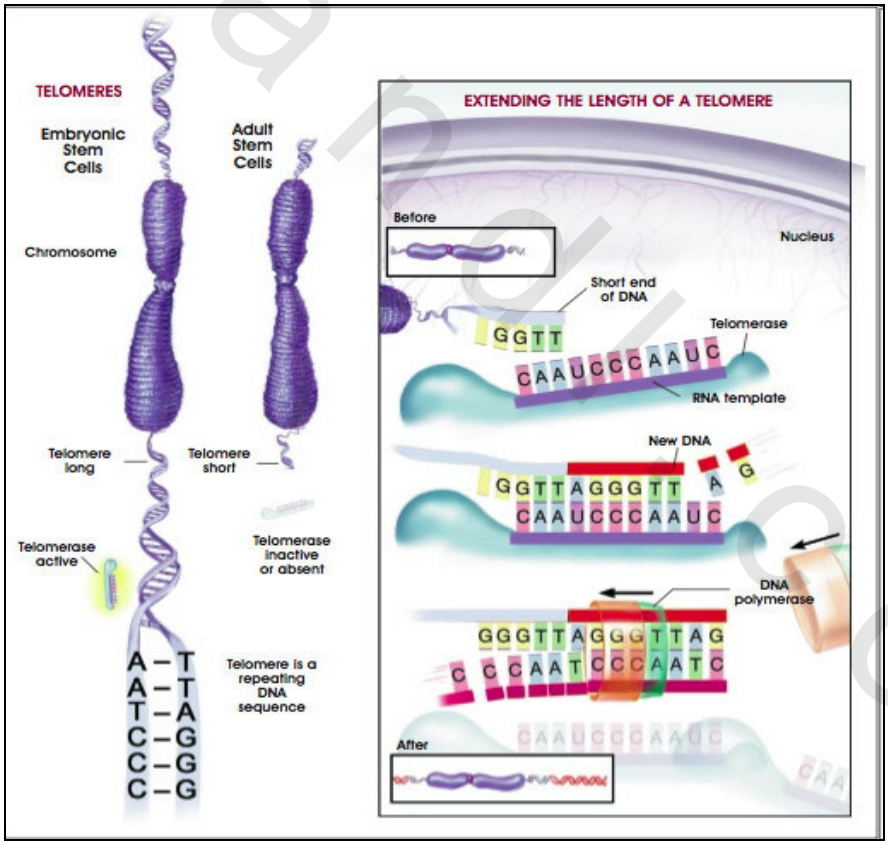

Fig. (2): Function and mechanism of action of telomerase.

Telomeres and telomerase complex of normal HSCs

The limited mean life span of mature blood cells requires their continuous production, which exceeds 10^{12} cells per day in adult individuals. Most HSCs and progenitor cells are in a resting phase (G0). The length of the telomeres in HSCs gradually shortens as a result of proliferation, differentiation and cell aging. It has been proved that stem cells which were obtained by the separation from bone marrow aspirate had shorter telomeres in comparison with cells from foetal tissues or possibly, blood cells from the umbilical vein [146]

Most circulating HSCs exhibit low telomerase activity. Conversely, lymphocytes in clonal expansion possess substantial telomerase activity. Thymic T-cells exhibit a much higher telomerase level in comparison with the same cells in lymph-nodes or in resting phase of the peripheral lymphocytes.Whereas telomere loss with ageing corresponded to 33 bp per year in T-cells.Telomere shortening was slower in B cells, corresponding to 15 bp/year. Separation of adult B-lymphocyte subpopulations based on CD27 expression revealed that telomere length was almost 2 kb longer in CD19+CD27+ (memory) compared with CD19+CD27- (native) cells (Martens et al., 2002). The elongation process is probably important for preservation of the long term B-cell immune response. The reduction of telomere length *in vivo* in peripheral lymphocytes is specific for B- and T-cells. [147,148]

The Role of Telomeres and Telomerase Complex in Haematological Neoplasia:

If telomeres are dysfunctional or lose the protective structure of the caps and thus cannot protectively elongate the chromosomal ends. They cause a response to DNA damage via the p53 and pRb/p16INK4a pathways. Those pathways induce either cell cycle arrest or apoptosis. If both control mechanisms of DNA damage are impaired (cells bypass barrier of mortality – M1 senescence), extensive shortening of telomeres with the continuing proliferation results in aberrant fusion of the unprotected chromosomal ends. This stage is identified as the secondary barrier of mortality (M2) or crisis. This period is characterized by extensive cellular death and chromosomal instability. A very low percentage of cells ($10–^{9}–10–^{5}$) can overcome the critical period in which the mutation and epigenetic changes activate telomere supportive mechanisms (telomerase activation and the ALT mechanism). [149]

The result of telomere protection is elongation of the proliferation period up to immortality of the cell. Investigations dealing with telomere length describe erosion of the telomere region, which correlates with the occurrence and severity of some hematopoietic diseases such as Aplastic Anemia (AA), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL). [150]

The hematopoietic cells of patients with AA exhibit shorter telomeres in comparison with age-matched control cells and also show correlation between the loss of telomeres and duration of the disease. Telomere erosion probably provides information on the disease stage. The telomere length in leukocytes in patients with AA, who have been treated by immunosuppressive therapy, does not differ from control leukocytes. Whereas significantly shortened telomeres have been detected in untreated patients as well as in patients without any response to therapy. In patients with MDS, only slightly increased

telomerase activity is observed in bone marrow cells. This is in contrast to very high level of telomerase activity in primary acute leukaemia. (150)

Significantly eroded telomeres have been detected in patients with MDS with an abnormal karyotype. Shortened telomeres have been associated with the disease progression and were correlated with specific chromosomal aberrations (deletion 5q, monosomy 7 and trisomy 8). (151)

Approximately 80% of patients in the chronic phase of CML exhibit a reduced telomere length and slightly increased telomerase activity. During CML progression into the acute and blastic phase, increased genome instability occurs in the vast majority of patients resulting in an increase of cytogenetic changes and thus increased telomerase activity and telomere shortening. (152)

The above mentioned facts suggest that telomere length can be regarded as a prognostic factor in patients with haematological malignancies.

Telomere/telomerase in transplant biology and telomere/anti-telomerase therapy

Hematopoietic reconstitution after allogeneic marrow transplantation relies on a relatively small number of HSCs compared with the estimated stem cell pool in the donor. It is reasonable to speculate that extreme proliferative demand on limited number of stem cells would result in significant telomere shortening. Furthermore, short telomeres might limit the cells' remaining replicative capacity. This may be of special significance after HSCT. Notaro et al. first demonstrated significant telomere shortening in human peripheral blood granulocytes of HSCT recipients as compared with their donors. (153)

Shortened telomere lengths have been found in various hematopoietic cell subsets after allogeneic HSCT. Telomere shortening in long-term survivors may be determined by the initial telomere loss during the repopulation period as well as by host-related factors such as chronic graft-versus-host disease (GVHD). Nevertheless, other studies on telomere loss after HSCT are desirable. (154)

New discoveries in telomere structure and the functions of telomere associated proteins have led to an increasing interest in targeting telomeres instead of telomerase in anti-cancer therapy. One strategy is to target telomere associated proteins that regulate telomere function, e.g. triggering telomere loss and inducing apoptosis or senescence through inhibition of the telomeric DNA-binding proteinTRF2. The 3' G-rich overhang can also fold into a 4-stranded DNA structure, termed G-quadruplex, so as to render telomeres inaccessible to telomerase action. Another strategy is to stabilize this G-quadruplex structure. (155)

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