Molecular and Cytogenetic Markers in Acute Myeloid Leukemia

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Abstract: Acute myeloid leukemia (AML) is a disease of hematopoietic progenitor cells with acquisition of heterogeneous genetic abnormalities that cause abnormal cell growth, proliferation and differentiation. Cytogenetic abnormality is considered an important prognostic factor in AML patients. AML patients are prognostically classified into three groups (favorable, intermediate, and poor) based on their molecular and cytogenetic analysis. Clonal chromosomes alterations are detected in approximately 50-55% of adults with AML. However, 40% to 49% of adults and 25% of children with AML, have no detectable chromosomal abnormality can be found on standard cytogenetic analysis. These cytogenetically normal (CN) patients have been classified as an intermediate-risk. Recently, with the advent of next generation sequencing, different molecular genetic abnormalities have been found in AML such as *DNMT3A*, *TET2*, *IDH1*/2, *NRAS*, *KRAS*, *BCOR*, *RUNX1*, and *WT1*. However, the significance of many of these gene mutations is unclear.

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1.Introduction

The diagnosis, prognosis, and treatment of acute myeloid leukemia (AML) has been transformed over the past 15 years from a disease defined and classified based on morphological characteristics alone to a disease classified largely based on genetic, and molecular characteristics. The current World Health Organization (WHO) 2008 classifies AML into favorable, intermediate and poor prognostic groups is based on cytogenetic and molecular genetic abnormalities.[7, 10- 18]. The risk pattern in AML is determined not only by cytogenetic abnormalities, such as chromosomal deletions, duplications, or substitutions, but also by the elucidation of certain molecular mutations leading to over- or under-expressions of one of many proteins. Cytogenetic studies performed on bone marrow in patients with AML play a crucial role in characterizing the leukemia, helping determine disease aggressiveness, response to treatment, and prognosis.

Molecular Pathology of AML

I. Cytogenetically normal AML:-

Almost half of AML patients (40-49%) have normal karyotype. Patients with normal-karyotype AML usually have an intermediate risk with a 5-year OS of 25% to 45%, but clinical outcome may vary greatly.[18- 23], the molecular changes in acute myeloid leukemia with normal cytogenetic include:

A. Mutations in Nucleophosmin (NPM1)

1. Physiological function:-

Falini *et al.* , in 2005 discovered abnormal cytoplasmic localization of NPM1.Normally it is located in the nucleoli and has multiple functions (Table 1). [1-23]

Table 1: NPM1 functions

Ribosome biogenesis.
Response to stress stimuli such as UV irradiation and hypoxia.
Maintenance of genomic stability.
Regulation of activity and stability of tumor suppressor genes such as *p53* and ARF.
Prevention of nucleolar protein aggregation and transport of these proteins.
Acts as a Co-repressor in retinoic acid (RA)–associated transcriptional regulation.

2. Molecular pathology:-

Cytoplasmic accumulation of NPM1 mutants is caused by two major alterations:

i -Loss of Tryptophan residues (290 & 288 or 290 only) normally required for NPM1 binding to the nucleoli.

ii - Generation of an additional nuclear export signal motif at the C-terminus by the exon 12 mutation causing its aberrant cytoplasmic localization [20- 28].

Moreover, NPM1 leukemic mutants recruit wild-type NPM1 from nucleoli to nucleoplasm and cytoplasm through dimerization. The most common *NPM1* mutation is 4–base pair duplication, 956dup TCTG in exon 12.

3. Clinical characteristics:-

NPM1 mutations can be identified in 45% to 62% of patients with CN-AML and thus are the most frequent genetic change in this patient subset. They appear to be less frequent in pediatric AML. Patients with NPM1 have different clinical characters compared to those who do not have this mutation (Table 4). [20 -28].

Table 2: Pretreatment characteristics of patients with CN-AML with NPM1 in comparison to those without NPM1 mutation

Female sex.
Higher bone marrow blast percentages.
Higher lactate dehydrogenase levels.
Higher white blood cell (WBC) and platelet counts.
High CD33-antigen expression.
Low or absent CD34-antigen expression.
Monocytic differentiation (FAB M5b).
FAB M1, M2, or M4 morphologies (children).

4. Clinical significances:-

Approximately 40% of patients with NPM1 have a mutation of FLT3-ITD.Patients with *NPM1* mutations lacking *FLT3*-ITD have significantly better CR, EFS, RFS, DFS, and OS, whereas *NPM1* mutations do not impact the poor outcome of patients having *FLT3*-ITD1-10. *NPM1* mutations usually precede the acquisition of *FLT3*-ITDs, suggesting that *NPM1* mutations might constitute a primary event in leukemogenesis [25- 28].

Approximately 11% to 15% of *NPM1* mutations occur outside the group of CN-AML, in combination with various recurring cytogenetic abnormalities (20-28).*CEBPA* mutations occur with a similar incidence in patients with and without *NPM1* mutations, whereas the partial tandem duplication (PTD) of *MLL* is extremely rare in patients with *NPM1* mutations [2].

5. Target Therapy:-

Patients with NPM1 mutation have good prognosis and no specific targeted therapy has been evaluated [6].

B. Mutations of the FMS-related tyrosine kinase3 *(FLT3)* genes

1. Physiological function:-

The *FLT3* gene is a class III receptor tyrosine kinase family as FMS, KIT. It is normally expressed on the surface of bone marrow (BM) hematopoietic progenitor cells and plays an important role in the survival, expansion and/or differentiation of multipotent stem cells [1, 3,4,10]. The first report demonstrating that internal tandem duplications (ITDs) within the juxtamembrane domain (JMD) of the *FLT3* gene are recurrent genetic alterations in AML was published in 1996. [1-23, 29-37]

2. Molecular pathology:-

In patients with AML, somatic mutations that result in the constitutive activation of FLT3 have been identified in two functional domains of the receptor (Table 3):-

i. The Juxtamembrane (JM) domain and
ii. The split TKD.[29-37].

Table 3: Comparison of JMD & TKD mutations

JM mutation	TKD mutation
The JM domain is disrupted by ITDs of various size and insertion sites in 28% to 34% of CN- AML, whereas JM domain point mutations are rare. *FLT3*-ITDs result in ligand-independent dimerization and tyrosine autophosphorylation as well as activation of RAS/MAPK, STAT5 and PI3K/AKT	The activation loop (AL) in the carboxy-terminal lobe of the TKD is affected by point mutations, small insertions, or deletions mainly involving codon 835 and 836 in 11% to 14% of CN-AML.
28-33% of CN-AML	Less frequent
Adverse prognostic factor	Not reported

3. Clinical characteristics:-

Patients with FLT3 mutations have different clinical characters than those without FLT3 mutations such as higher white blood cell counts (WBCs), higher percentages of blood and BM blasts and more with de novo than secondary AML.

4. Clinical significances:-

Patients with CN-AML harboring *FLT3*-ITD have a significantly inferior outcome compared to patients without *FLT3*-ITD.This are true for CRD, CIR, DFS, EFS, RFS and OS. Further analysis by Whitman *et al.,* and Thiede *et al.,*shows that only patients having lost wild-type *FLT3* have a dismal outcome and not those with heterozygous mutations and cases with an *FLT3*-mutant/*FLT3*–wild-type allele ratio above the median value had a significantly shorter OS and DFS compared with patients without FLT3-ITD[18- 23].

Thiede *et al.,* were able to identify a threshold that distinguished prognostic subgroups. Prognostic impact of the length of the *FLT3*-ITD has been shown to be significant as patients with a duplicated segment consisting of at least 40 nucleotides had the worst relapse-free survival (RFS) compared with patients with smaller duplications and those without *FLT3*-ITD [1-8,31-39].The *FLT3*-TKD has not been reported to have an adverse prognosis among either CN patients or those with intermediate-risk cytogenetic [1-23, 29-37].

5. Targeted therapy:-

In patients with *FLT3*-ITD or *FLT3*-TKD mutations, clinical responses are usually transient with few patients attaining a CR or a long-lasting partial response. Nevertheless, *in vivo* inhibition of FLT3 autophosphorylation by PKC41267 (Midostaurin), CEP-70168 (Lestaurtinib) and SU11248 (Sunitinib) has been demonstrated in responding patients, supporting the further clinical development of these compounds [1-25, 31-39].

Notably, all these compounds are also capable of inhibiting multiple tyrosine kinases, which may also explain clinical responses in AML patients without *FLT3*-ITD.Combination of FLT3 inhibitor with conventional chemotherapy needs further investigations [29-37].

C. Mutations of the CCAAT/enhancer-bindingProtein A *(CEBPA)* gene

1. Physiological function:-

The transcription factor CEBPA is an essential molecule in the mediation of lineage specification and differentiation of multipotent myeloid progenitors into mature neutrophils [10-15].

2. Molecular pathology:-

There are 2 main categories of *CEBPA* mutations:

i- C-terminal mutations that occur in the bZIP domain (basic region-leucine zipper domain), which are usually in-frame and predict mutant proteins lacking DNA binding and/or homodimerization activities; and

ii- N-terminal nonsense mutations that prevent expression of the full-length protein and result in truncated isoforms with dominant negative activity [19- 23, 38-42].

3. Clinical characteristics:-

Point mutations of the CEBPA gene at chromosome 19q31.1 are detected in 13% of CN-AML cases in adults and 17% to 20% of CN-AML cases in children 6.Approximately 70% of AML patients with CEBPA mutations have a normal karyotype. Approximately 10% have a single karyotypic abnormality, and only rare cases have a complex karyotype.clinically these patients are different (Table 4), [17—22].

Table 4:Pretreatment characteristics of patients with CEBPA mutation in comparison to those without CEBPA mutation

Higher percentages of blood blasts.
Lower platelet counts.
Less lymphadenopathy and extramedullary involvement.
Most commonly FAB M1and M2, with fewer cases of M4 and M5.
Rare in Therapy-related AML.

4. Clinical significances:-

Clinical studies revealed that *CEBPA* mutations were associated with a significantly better CRD, EFS, DFS, and OS among patients with CN AML [1-10]. At diagnosis, patients with *CEBPA* mutations had less frequent *FLT3*-ITD and *FLT3*-TKD, and no *MLL*-PTD. CR rates did not differ between patients with and without *CEBPA* mutations. Fro¨hling *et al.,* also analyzed clinical outcome separately for patients with N-terminal nonsense *CEBPA* mutations, those with other *CEBPA* mutation types, and those with wild-type *CEBPA* and found that the CRD of patients with N-terminal mutations was the longest, followed by CRD of patients with other mutations and of those without *CEBPA* mutations [1, 20-25, 38-42]

5. Targeted therapy:-

In the absence of adverse prognostic factors, AML with CEBPA mutations has a favorable prognosis not likely to benefit from allo-SCT, although further study is needed to address optimal

postremission therapy, because the number of cases studied is too small to be definitive [38-42].

D. Partial tandem duplication (PTD) of the Myeloid/lymphoid or mixed-lineage Leukemia *(MLL)* gene

1. Physiological function:-

MLL is an activator protein that through AT hook motifs can associate with specific DNA sequences.MLL gene has multiple functions that are retained by mutated MLL-PTD gene [2- 20] (Table 5).

Table 5:- Functions of MLL

Required for Hoxc8 expression.
Binds and Methylates histones at the Hox loci (at histone H3 lysine 4).
Links the N terminal region of the protein, which contains the DNA recognition region to one of dozens of partner proteins of diverse functions.
Dimerize with themselves and wild-type MLL.
Prevents the acetylation and activation of P53, that can affect cycle, apoptosis and genomic stability.

2. Molecular pathology:-

MLL-PTD result in a duplication of a genomic region spanning exons 5 through 11(rarely the duplicated region encompasses exons 5 through 12) and insertion of the duplicated region into intron 4 of the *MLL* gene [43-46]. Both main types of *MLL*-PTD result in in-frame fusions producing an elongated protein. The presence of *MLL*-PTD is concurrent with silencing of the *MLL* wild-type allele in AML blasts [1-23].

3. Clinical characteristics:-

Among CN-AML patients, *MLL*-PTD occurs in approximately 5%-11%.Translocations involving the MLL gene on chromosome 11q23 are found in approximately 6% of cases of AML and are associated with more than 50 different partner genes. Pretreatment characteristics do not differ significantly between CN patients with and without *MLL*-PTD [1, 43-46].

4. Clinical significances:-

CRD, RFS and EFS are worse for patients with CN AML. There is no significant difference in the probability of achieving a CR or OS. Between 30% and 40% of *MLL*-PTD–positive patients also harbor *FLT3*-ITD, whereas coexistence of *MLL*-PTD with *CEBPA* or *NPM1* mutations is rare [1-23, 43-46]

5. Targeted therapy:-

Whitman et al have shown that the combination of Decitabine (a DNA methyltransferase inhibitor), and Depsipeptide (a histone deacetylase inhibitor), can induce MLLwild-type allele which consequently associated with enhanced cell death of MLL-PTD–positive leukemic blasts. However, until molecularly targeted therapies, allogeneic SCT appears to be the best therapeutic option for CNAML patients with *MLL*-PTD [1-23, 43-46].

E. Overexpression of the ETS-related Gene *(ERG)*

1. Physiological function:-

The *ERG* gene, a member of the *ETS* family of transcription factors, which are involved in regulation of cell proliferation,differentiation, and apoptosis [3-23].

2. Clinical characteristics:-

No significant clinical characteristics between patients with and without overexpression of ERG gene.

3. Clinical significances:-

In CALGB 9621 adults younger than 60 years with de novo AML, whose high *ERG* expression in pretreatment blood placed them in the uppermost quartile of *ERG* expression, had a significantly worse CIR and OS than patients in the 3 quartiles with lower *ERG* expression. In multivariable analyses, high *ERG* expression and *FLT3*-ITD independently predicted worse CIR and OS [1-11, 47- 49].

F. Overexpression of the *BAALC* gene

1. Physiological function:-

The *BAALC* gene is expressed primarily in neuroectoderm-derived tissues and hematopoietic precursor cells only [1-23, 47].

2. Clinical characteristics:-

No clinical differences between patients with high BAALC expression and those with low BAALC expression.

3. Clinical significances:-

In CN adults younger than 60 years with de novo AML, high *BAALC* expression blood or bone marrow at diagnosis negatively affected EFS, DFS, and OS [1-14]. A subgroup analysis, restricted to patients without *FLT3*-ITD or *CEBPA* mutations, also showed high *BAALC* expression to be associated with worse DFS and OS. Bienz *et al.,* concluded that *BAALC* expression appears to be particularly useful as a prognostic marker in CN AML patients lacking *FLT3*-ITD and *CEBPA* mutations. On multivariable analysis, high expression of *BAALC* in blood and high *FLT3*-ITD/*FLT3*–wild-type ratio were independent adverse prognostic factors for resistance to initial induction chemotherapy, high CIR, and shorter OS [1, 20- 23, 47].

4. Targeted therapy:-

Allogeneic SCT in first CR might overcome the higher relapse rate associated with high *BAALC* expression compared with ASCT or chemotherapy [1-23].

G. Mutations in RAS, WT1

NRAS mutations occur in 9-14% of adult patients with CN-AML [2, 7]. Till now, there is no prognostic impact of *NRAS* mutations in either CN-AML patients or in those with intermediate-risk cytogenetic [2, 7, 8, 9].Mutations in Wilms' Tumor 1 gene (*WT1*) in AML were first reported by King-Underwood and Pritchard-Jones in 1998.Disruption of WT1 function by mutation of the gene could either promote proliferation or induce a block in differentiation. In a study by Summers *et al.,WT1* mutations were detected in 10% of the patients. Mutations consisted of insertions or deletions that mainly clustered in exons 7 and 9. WT1 mutations may be associated with induction failure [2, 7, 8, 9, 50-52].

H. Deregulated Gene Expression

Over expression of a number of genes, such as *MN1* or *EVI1,* has been shown to be of prognostic significance [2, 3, 7-9, 51].The Meningioma 1 (*MN1*) gene has been found rearranged in a t (12; 22)(p13;q11-12), a balanced translocation in AML leading to a fusion of *MN1* with *ETV6*. Using global gene expression profiling, *MN1* was associated with induction failure, a higher relapse rate and inferior RFS and OS in patients with AML (2, 3, 7-9).

EVI1 overexpression has been shown to be associated with inferior outcome in patients with intermediate-risk cytogenetic (2-4, 7-9, 51)

I. Other prognostic factors in karyotypically normal AML

Overexpression of breast cancer resistance protein (BCRP) encoded by the *ABCG2* gene is associated with the worst DFS, shortened CRD, and high induction failure rate [1-14, 53].

Table 6: Summary of molecular changes in CN AML and their significances

Good molecular changes	Bad molecular changes	unknown
NPM1 CEBPA	FLT3 BALAC MLL KIT ERG WT1 MN1 EVI1 ABCG2	NRAS FLT3-TDK

II. Acute myeloid leukemia with balanced translocations/inversions

The different subtypes of leukemia in this category have characteristic clinical features with heterogeneous prognostic implications.

A. Core-Binding Factor (CFB) AML

CBF AML is defined by the presence of the t(8;21)(q22;q22) or the inv(16)(p13q22)/t(16;16)(p13;q22). Mutations have been identified in the genes encoding KIT, FLT3, NRAS, KRAS, and JAK2.[1-14]

I- Acute Myeloid Leukemia with t(8;21)(q22;q22):-

Molecular Pathology:-

The translocation results in a fusion product involving the RUNX1 gene (Core-Binding Factor Alpha (CBFα) and AML1) on chromosome 21 and the RUNX1T1 (ETO) gene on chromosome 8 [1-14].

Clinical characteristics:-

AML with t (8; 21) is common in children and adults, accounting for approximately 8% of the cases of AML. The presence of this genetic abnormality is diagnostic of AML regardless of the blast count. Cases of AML with t (8; 21) share a common morphology and immunophenotype (Table 7).[1-10, 54-58].

Table 7: Morphological characteristics of patients with t(8,21):-

The blasts are of myeloid maturation (FAB M2).
The maturing neutrophils are commonly dysplastic.
Background Eosinophilia is variably present.
Immunophenotypically, blasts express CD34, CD13, CD33, and Myeloperoxidase (MPO), with typical aberrant weak CD19 expression.
Other B lineage antigens may be found, such as PAX5, CD79a, and TdT.

Clinical significances:-

Core-binding factor leukemia's are associated with a favorable prognosis in children and adults, especially when treated with repetitive cycles of high-dose Cytarabinepostremission. [1-15, 54-58]. Mutations of KIT in core-binding factor AML are common (20%–25%).In adults, KIT mutations in exons 8 and exon 17 appear to worsen prognosis. It is unclear if patients with t (8; 21) AML with KIT mutation will benefit from Allogenic-SCT in first remission [1-10, 54-58].

Mutations in FLT3 are rare in core-binding factor leukemia. Additional cytogenetic abnormalities are present in most cases of t (8; 21) AML, most

commonly including loss of a sex chromosome or partial deletion of the long arm of chromosome 9. The presence of an unfavorable additional cytogenetic abnormality, such as monosomy 7, may adversely impact prognosis[1-15, 54-58].

II- Acute Myeloid Leukemia with inv (16) (p13; q22) or t (16; 16) (p13; q22):-

Molecular Pathology:-

The genes at the break point junction are the beta subunit of the core-binding factor (CBFB) at 16q22 and a gene encoding smooth muscle myosin heavy chain (MYH11) at 16p13 (1-23, 54-58).

Clinical characteristics:-

AML with an inv (16) (p13; q22) or t (16; 16) (p13; q22) comprises 10% of adult AML and approximately 6% of childhood AML. The presence of this genetic abnormality is diagnostic of AML, regardless of the blast count. Clinically these patients have specific characteristics (Table 8) [1-23].

Table 8: Pretreatment characteristics of patients with inv(16) in comparison to those without inv(16)

High incidence of extramedullary disease
High incidence of central nervous system relapses.
Morphology of acute myelomonocytic leukemia with abnormal eosinophils in the bone marrow (FAB-M4Eo).
Immunophenotypically typically reveals multiple populations, including an immature blast population expressing CD34 and/or CD117 and groups of cells exhibiting granulocytic (CD13, CD33, CD15, MPO) or monocytic (CD4, CD11b, CD11c, CD14, CD64, CD36, Lysozyme) differentiation.
Aberrant Coexpression of CD2.

Clinical significances:-

Patients with inv (16) or t(16,16) have good prognosis.KIT mutations are present in approximately 30% of cases and negatively affect prognosis in adults. It is not clear if AML with inv (16) or t (16; 16) and mutated KIT benefits from Allogenic-SCT or tyrosine kinase inhibitor therapy [1-15, 54-58].

B-Acute Promyelocytic Leukemia with t (15; 17) (q22; q12):-

Molecular pathology:-

The t(15;17)(q22;q21) results in fusion of the promyelocytic gene (PML) on chromosome 15 with the retinoic acid receptor (RARA) gene on chromosome 17 [1-15, 59].

Clinical characteristics:-

Acute promyelocytic leukemia (APL) usually presents with an abrupt onset and comprises 5% to 8% of cases of AML. It is most common in young adults. Prompt recognition of the diagnosis is essential because of the high frequency of life-threatening disseminated intravascular coagulation (DIC). Detection of this abnormality is diagnostic of APL regardless of the blast count. Two morphologic variants (hypergranular and hypogranular) are recognized to have distinct immunohistochemical and genetic features (Table 9) [1-15, 59].

Table 9:Morphological variant of APL

	Hypergranular (typical) variant	Microgranular variant
Frequency	60-70%	Less
WBC count	Low	High
Morphology	Numerous large red to purple granules with blobbed nuclei Multiple Auer Rods	Fine granules with blobbed nuclei Less Auer Rods
Flowcytometry	Lack HLA-DR & CD34 Bright CD33,MPO Absent CD15	Dim HLA-DR & CD34 Bright CD13,CD33,MPO Variant CD64 Variable CD2 & CD56
Clinical Significance	Less FLT3-ITD	More FLT3-ITD
Break point	Bcr1,2	Bcr3

Clinical significances:-

Cytogenetic analysis, fluorescent in situ hybridization (FISH), or RT-PCR is necessary for genetic confirmation of the PML-RARA fusion. FLT3 mutations are common in APL and are present in approximately 40% of patients, with the majority being internal tandem duplication (ITD) mutations [1-15, 59].

Targeted therapy:-

The PML-RARA fusion protein mediates a block in myeloid differentiation, which can be overcome using ATRA therapy and Arsenic trioxide

(ATO). Standard induction chemotherapy with high-dose anthracyclines is given in addition to ATRA. The prognosis is better than for any other category of AML. Initiation of therapy should not wait for genetic confirmation when other investigations are consistent with the diagnosis of APL [1-15,59]

Acute Promyelocytic Leukemia with Variant RARA Translocations:-

Uncommonly, variant cytogenetic translocation that involves the RARA gene on chromosome 17 but not the PML gene on 15 has been described (Table 10). These cases are important to recognize, because some variants, including ZBTB16-RARA and STAT5B-RARA,do not respond to ATRA therapy [1-15].

Table 10: APL with Variant RARA translocations

1- t (11; 17) (q23; q12) ZBTB16-RARA.
2- t (11; 17) (q13; q12) NUMA1-RARA.
3- t (5; 17) (q35; q12) NPM1-RARA.
4- t (17; 17) (q11.2; q12) STAT5B-RARA.

C-Acute Myeloid Leukemia with t (9; 11) (p22; q23):-

The 2008 WHO classification now recognizes only t (9; 11) (p22; q23) (MLLT3-MLL) as a specific entity in the category of AML with recurrent genetic abnormalities [1-17].

Table 11:Clinical Features of AML with t (9; 11) (p22; q23)

Extramedullary disease of the gingival and skin.
Presentation with DIC may occur.
The blasts typically have Monocytic or Myelomonocytic morphology.
Typically MPO negative.
In children, AML with t (9; 11) typically expresses CD33, CD4, CD65, and HLA-DR, with minimal to no CD13, CD14, and CD34 expression.
In adults, AML with 11q23 translocations may express CD14, CD64, CD11b, CD11c, and CD4.
CD34 is often negative, with variable CD117 and CD56 reactivity.

Clinical characteristics:-

Translocations involving the MLL gene on chromosome 11q23 are found in approximately 6% of cases of AML and are associated with more than 50 different partner genes. Clinically they are different (Table 11) [1-17].

Clinical Features:-

The clinical features are summarized in table 11.

Clinical significances:-

The t (9; 11) (MLLT3-MLL) AML typically occurs in children, with an intermediate prognosis. Approximately 20% of AML with t (9; 11) have activating loop domain point mutations in FLT3.Pediatric AML with t (9; 11) has an intermediate prognosis. Overexpression of ectopic virus integration-1 (EVI1) has been described in multiple variant translocations of 11q23 and has been associated with a poor prognosis [1-16].

D-Acute Myeloid Leukemia with inv (3) (q21q26.2) or t (3; 3) (q21; q26.2)

Clinical characteristics:-

AML with inv (3) (q21q26.2) or t (3; 3) (q21; q26.2) RPN1-EVI1 occurs primarily in adults, representing 1% to 2% of cases of AML. Rare cases are reported in children in association with monosomy 7. Patients with inv (3) or t (3; 3) may present with less than 20% blasts and should be closely monitored for the development of AML (Table 12) [1-16, 60, 61].

Table 12: Clinical characteristics of AML with inv (3) or t (3; 3)

Patients typically present with anemia, and sometimes thrombocytosis, with or without prior MDS. Hepatosplenomegaly may be present.
The peripheral blood and BMA may show dysplastic erythrocytes, neutrophils and platelets.
Morphology includes FAB M1, M4, and M7 types.
Myeloperoxidase activity is often low.
Megakaryocytes may be normal or increased in number, usually with small unilobated and bilobated forms or other dysplastic features.
The core biopsy may show decreased cellularity and occasionally fibrosis.
Blasts typically express CD34, CD13, CD33, and HLA-DR, with aberrant CD7 expression in some cases. Cases with megakaryocytic differentiation may express CD41 and CD61.

Clinical significances:-

EVI1 is abnormally expressed in AML with inv (3) (q21q26.2) or t (3;3)(q21;q26.2). High expression of EVI1 is a poor prognostic indicator independent of 3q26 rearrangement. Cytogenetic may fail to identify cryptic rearrangements of 3q26 detectable by FISH. Secondary karyotypic abnormalities are present in most cases, including most of those with poor prognosis, MDS-associated abnormalities of -7, -5q, and complex aberrant karyotypes. FLT3-ITD mutations are found in a small subset of patients (13%). Patients with AML with inv (3) or t (3; 3) typically have short survival [1-16, 60, 61].

Targeted therapy:-

Patients able to tolerate Allogenic-SCT may benefit from this approach to therapy.

E-Acute Myeloid Leukemia with t (6; 9) (p23; q34):-

Clinical characteristics:-

AML with t (6; 9) is a rare subtype of AML comprising approximately 1% of cases in children and adults. The median age in adults for this subtype of AML is young (35 years). The presence of fewer than 20% blasts in a patient with the t (6; 9) (p23; q34) is not considered diagnostic of AML, and patients should be followed closely for progression. Clinical characters are shown in (Table 13) [1-16, 62-64].

Table 13: Clinical characteristics of AML with t (6; 9) (p23; q34):-

Occasional Auer rods
Monocytic features.
Dyspoiesis of all three lineages.
Basophiles (>2% marrow or blood basophiles) is present in roughly half of reported cases.
Blasts typically express CD45, CD13, CD33, HLA-DR, and intracytoplasmic MPO, with variable expression of CD34, CD15, and CD11c.
TdT may be positive in some cases by Flowcytometry or Immunohistochemistry.

Clinical significances:-

FLT3-ITD mutations are common in this type of AML, with a reported frequency of 70%. Although the majority of patients with t (6; 9) AML may achieve CR, survival rates are poor with conventional chemotherapy [1-16].

Targeted therapy:-

Patients may benefit from Allogenic-SCT. [1-8, 62- 64]

F-AML (Megakaryoblastic) with t (1; 22) (p13; q13):-

Clinical characteristics:-

Acute megakaryoblastic leukemia (AMkL) with t (1; 22) is a rare form of infantile AML. The median age at diagnosis is 4 months, and 80% of cases are diagnosed in the first year of life. AMkL with t(1;22) composes approximately 1% of childhood AML. Cases commonly present with less than 20% blasts in the blood and/or marrow, but the presence of myeloid sarcoma is diagnostic of AML regardless of the marrow blast count (Table 14) [1-16].

Table 14: Clinical characteristics ofAML with t (1; 22) (p13; q13)

May present as a solid tumor with hepatosplenomegaly and/or skeletal lesions (bilaterally symmetric periostitis and osteolytic lesions), sometimes without involvement of the bone marrow.
Biopsy of the lesions may show cohesive nests of small round blue cells also suggestive of a childhood solid tumor, leading to a misdiagnosis.
The complete blood count may show anemia and thrombocytopenia.
Blasts in the blood and/or bone marrow show typical features of megakaryoblasts with a small amount of agranular cytoplasm typically with budding or blebs.
Marrow fibrosis may be so extensive.
Micromegakaryocytes are reportedly common.
CD45 and CD34 may be negative,
Myeloid antigens CD13 and CD33 are inconsistently expressed, as is HLA-DR.
Megakaryocytic antigens, CD41 and CD61, are commonly seen, and some cases may express CD56.
Markers of megakaryocytic differentiation, such as von Willebrand factor, may be positive.

Clinical significances:-

The diagnosis of a myeloid sarcoma AMkL with t (1; 22) may not be obvious until cytogenetic reveals the presence of the translocation. ‘‘Older’’ patients (greater than 6 months of age) commonly have complex additional karyotypic abnormalities. The frequency of FLT3 mutations is unclear, given the rarity of AMkL with t (1; 22) [1-17].

Targeted therapy:-

AMkL with t (1; 22) was associated with poor survival in earlier studies, but more recent studies suggest that they respond well to intensive AML therapy [1-17].

III. Acute myeloid leukemia with MDS-related changes:-

AML with MDS-related changes (AML-MRC) may present as a de novo disease or may evolve from an earlier (MDS) or Myelodysplastic/Myeloproliferative disorder (MDS/MPD) [1-10].

Clinical characteristics:-

The inclusion criteria include AML arising from a previous MDS or MDS/MPD; AML with a specific MDS-associated cytogenetic abnormality, and/or AML with Multilineage dysplasia.

The exclusion criteria are patient should not have history of prior cytotoxic therapy and not have one of the recurring cytogenetic abnormalities of AML with recurrent genetic abnormalities [1-10, 66, 67].The blast count must be 20% or more in the blood or marrow. Morphologically AML-MRC should have an evidence of dysplasia in 50% or more of developing cells in two or more lineages [1-10, 65,66].

Molecular characteristics and their clinical significances:-

MDS-associated chromosomal abnormalities are commonly high-risk changes. Recent data suggest that autosomal monosomies are a better marker of poor prognosis than complex karyotype alone. It appears that most AML cases with the ‘‘monosomal karyotype’’ would be grouped with AML-MRC because they almost all have complex karyotypes, but not all cases of AML with complex karyotypes have the ‘‘monosomal karyotype’’ [1-15].

All of the nine balanced cytogenetic rearrangements of AML-MRC are also seen in therapy-related myeloid proliferations. Multilineage dysplasia may not be detected in up to half of the cases with MDS-associated cytogenetic abnormalities. It has been observed that patients with MDS-associated cytogenetic abnormalities have a worse prognosis than those with multilineage dysplasia lacking the cytogenetic abnormalities.The morphologic designation seems to be useful; however, patients with a normal or intermediate risk karyotype and multilineage dysplasia have worse outcomes compared with AML-NOS. Some patients with AML-MRC characterized by multilineage dysplasia may have normal cytogenetic findings or a cytogenetic abnormality not related to the AMLMRC category or recurrent genetic abnormality diagnosis. These patients may have mutations of FLT3, NPM1, or CEBPA that may affect their prognosis. The presence of these mutations, in addition to the designation of AML-MRC (multilineage dysplasia), should be included in the diagnosis. The prognosis of AML-MRC is typically unfavorable. In patients with lower blast counts and multilineage dysplasia (20%–29% blasts), disease progression may behave more like MDS; this is especially true in children, who show a slower course of disease progression. Patients with MDS-associated cytogenetic abnormalities have a more consistently poor prognosis; especially those with a monosomalkaryotype. Patients with normal cytogenetic findings, mutated NPM1, and wild type FLT3 may have a more favorable course, although the data on this combination of mutations in this category are limited [1-10, 65, 66].

Table 15: Frequent Cytogenetic and Molecular Changes in AML-MRC

Complex karyotype (three or more unrelated abnormalities)
-Unbalanced abnormalities:-
-7/del(7q)
-5/del(5q)
i(17q)/t(17p)
-13/del(13q)
del(11q)
del(12p)/t(12p)
del(9q)
idic(X)(q13)
Balanced abnormalities:-
t(11;16)(q23;p13.3)
t(3;21)(q26.2;q22.1)
t(1;3)(p36.3;q21.1)
t(2;11)(p21;q23)
t(5;12)(q33;p12)
t(5;7)(q33;q11.2)
t(5;17)(q33;p13)
t(5;10)(q33;q21)
t(3;5)(q25;q34

IV. Therapy-related myeloid neoplasms

Therapy-related AML and MDS (t-AML/MDS) remain in a separate category, with the additional recognition of myelodysplastic/myeloproliferative overlap syndromes that can occur after cytotoxic therapy(Table 16). Therapy-related myeloid neoplasm comprises roughly 10% of AML and 20% of MDS cases. The incidence is increasing as more patients survive cancer [1-10, 67].

Clinical characteristics:-

Some patients may have karyotypic changes identical to de novo AML, including favorable prognosis core-binding factor leukemias or acute promyelocytic leukemia. The prognosis of therapy-related myeloid neoplasm is poor in general, with

overall survival reported at less than 10%. Patients with monosomy 5 and/or 7 karyotypes have a particularly dismal prognosis, with a median survival of less than 1 year, regardless of the blast percentage. Treatment is limited by toxicities of prior treatment and a drug resistance mechanism in the neoplastic cells. Treatment-related mortality is very high for intensive therapy. Allo-SCT may be the patient's best chance for a cure, but with significant short- and long-term mortality [1-10, 67].

Table 16:t-AML/MDS

Alkylating related t-AML/MDS	Topoisomerase related t-AML/MDS
The longer latency cases (5–7 years after therapy)	Shorter latency cases arise 1 to 3 years after therapy and comprise 20% to 30% of t-AML/MDS
They usually present with MDS with typical cytopenias and morphologic multilineage dysplasia.	Same
The bone marrow may be hypercellular, normocellular, or hypocellular, and may have associated fibrosis.	Same
Are commonly associated with chromosomal losses, often of chromosomes 5 and 7, in a setting of a complex karyotype, similar to AML-MRC.	Often have chromosomal translocations involving the MLL gene at 11q23 or RUNX1 at 21q22

V. Acute myeloid leukemia, not otherwise specified

FAB-like terminology is retained for morphologic description of cases lacking a recognized recurrent genetic abnormality, criteria for AML-MRC, a history of prior therapy, or Down syndrome. The epidemiology and clinical prognosis of these categories in the modern classification is unknown, because good and poor prognostic groups have been removed from the earlier, more expansive FAB categories. Correlation with cytogenetic and molecular genetics status is essential to provide the most clinically meaningful diagnosis [1-10, 68].

Conclusion:-

As molecular characterization becomes a more routine part of the diagnostic workup of AML, there is an imperative to determine whether different therapeutic approaches might be preferable for subgroups of patients. Developing a non-empiric approach to the many subtypes of AML that are being molecularly defined is a formidable scientific, administrative and biostatistician task. The recent focus on AML stem cell research is certainly exciting in this regard, although not unexpectedly, this biology has become more and more complex as our knowledge base has increased.

Although stem cell transplantation is not a 'targeted' therapy, it represents the major alternative for patients felt to have chemotherapy resistant disease, albeit with limitations posed by patient age and performance status There are however, limitations on what could be learned from these trials because of the relatively small number of patients, the absence of a discrete hypothesis explaining these findings, and the retrospective nature of the analyses.

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