



SJMLS-3(3)-009

Differential Molecular Diagnosis in Acute Myeloid Leukaemia: A ReviewAjayi O.I.¹, and Garba, N. *²

Department of Human Physiology, Faculty of Basic Medical Sciences, University of Benin, P.M.B.1154, Benin City, Nigeria ¹, Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Bayero University. P.M.B. 3011, Kano, Nigeria ²

Author for Correspondence*: ngarba.mls@buk.edu.ng/+234-803-336-4865

Abstract

Acute myeloid leukemia (AML) is heterogeneous clonal disorders characterized by the production of immature and non-functional myeloid cells known as blasts and subsequently result in bone marrow failure and organs infiltrations. This review aims at presenting a detailed account on differential molecular diagnosis in acute myeloid leukemia. Specifically, the differential karyotype in AML and its prognostic importance are discussed. The review also focused on the current gene mutations on all forms of AML. Cytogenetically, AML is divided into two; AML with abnormal karyotype and AML with normal karyotype. Cytogenetics testing remains a critical diagnostic and prognostic marker in AML with abnormal karyotype. But conventional cytogenetics analysis cannot identify any chromosomal aberration in AML with normal karyotype. This is because, there are some chromosomal aberrations that are generally cryptic and cannot be identified by conventional cytogenetics (AML with normal karyotype). Advanced techniques such as Array comparative genomic hybridization (array CGH), Nano string technology and gene sequencing are usually used for the identification of those aberrations. Differential molecular diagnosis had provided good information for both diagnosis and prognosis of AML. Chromosomal aberrations such as *t* (8;21), *t* (15;17) and *inv* (16) or *t* (16;16) confirm the presence of AML regardless of the blasts count and are associated with favorable prognosis. A newly diagnosed patient with AML and a normal karyotype should be checked for more common mutations on genes like *FLT3*, *NPM1*, *CEBPA*, *RUNX1*, *MLL-PTD*,

or *EVI 1*, which may have prognostic and possibly therapeutic implications. We therefore, recommend that, researchers should discover the driving oncogenic events such as gene mutations or other aberrations in cytogenetically normal AML (CN-AML) which will help to find targets for the development of novel therapies and to determine prognostic factors for this group of patients.

Keywords: Acute myeloid leukemia, abnormal karyotype, normal karyotype, Nano string technology, array CGH.

Introduction

The term, leukemia was described by Rudolf Virchow for the first time in 1856 (Piller, 2001). Acute myeloid leukemia (AML) is a form of malignant disorder that is characterized by uncontrolled and exaggerated growth of immature cells known as blast cells of myeloid origin (Nelson, 2008). AML is a clonal disorder characterized by the accumulation of somatically acquired genetic alterations in haematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation, and differentiation (Frohling *et al.*, 2005).

It is characterized by the abnormal growth of immature white blood cells (WBCs) that have lost their ability to differentiate and undergo apoptosis. These immature blood cells accumulate in the bone marrow, and interfere with the production of healthy blood cell (Deschler, B. and Lubbert, M, 2006). This decreases the number of platelets, red blood cells (RBCs) and normal WBCs, leading to a variety

of symptoms. These include fatigue and shortness of breath caused by anaemia, infections caused by neutropenia, and easy bruising and bleeding caused by thrombocytopenia (Redaelli *et al.*, 2003).

AML is the most common among acute leukemia affecting adults and its incidence increases with age even though AML is relatively rare disease, accounting for about 1.2% of cancer death in United States and its incidence is expected to increase as the population ages. There is no direct cause of AML, although there are a variety of risk factors that have been associated with AML. These risk factors include genetic predisposition which is reported in conditions like Fanconi anaemia, trisomy 8 and Down syndrome. Then environmental exposure to ionizing radiation and organic solvents like benzene also progression of pre-existing blood disorders such as myeloproliferative neoplasms (MPN) and myelodysplastic syndromes (MDS) can result in secondary AML (sAML), or prior cytotoxic chemotherapy for a different malignancy can cause therapy-related AML (tAML) (Hahm, 2015).

The diagnosis and prognoses of AML are determined by morphology, immunophenotyping, cytogenetics and molecular analysis. The cytogenetic studies are performed on bone marrow of patient which will consequently be used to characterize the leukaemia by which will help in knowing the degree of aggressiveness, prognosis and also response to therapy. Molecular studies also help in prognoses as it determines the molecular mutation that occurs in some genes which leads to their over or under expression.

The aim of this review is to present details about differential molecular diagnosis in acute myeloid leukaemia.

Methodology

The literature was obtained through a thorough electronic searching of articles, e-books, and reports. Textbooks and journals from the Medical Library of the College of Health Sciences, Bayero University Kano were also searched, used and well referenced for the purpose of this review.

Findings

Based on karyotype status, two major groups of AML can be distinguished: those with an abnormal karyotype, which accounts for approximately 52% of patients, and those who demonstrate a normal karyotype by conventional cytogenetics, which accounts for 48% of AML patients (Ronald *et al.*, 2013). But all of them present with gene mutations.

Acute myeloid leukaemia with Abnormal Karyotype

The mutations observed in AML range from relatively large genetic alterations, such as gains, losses, and translocations of chromosomes, to single nucleotide changes. Detection of many of these mutations is required for accurate diagnosis, prognosis, and treatment of patients with AML (Wertheim *et al.*, 2012).

An abnormal number (aneuploidy) or structure (pseudodiploidy) of chromosomes or both are evident in approximately 60% of AML. The most prevalent abnormalities are trisomy 8, monosomy 7, monosomy 21, trisomy 21, and loss of an X or Y chromosome (Kenneth *et al.*, 2010). The recently proposed World Health Organization (WHO) classification attempts to associate the prognostic variance with cytogenetic abnormalities. Specific translocations such as (8; 21) in AML-M2, t(15;17) in AML-M3 and inv(16)/t(16;16) have been documented in AML-M4eo. It has been described that a proportion of AML-M5 patients carry 11q23 (MLL gene) rearrangement with a wide spectrum of recurrent translocation partner chromosomes (Rui *et al.*, 2014). Cytogenetic studies of patients in the pediatric age group documented that the t(4;11), t(11;19), and 14q32 rearrangement may be frequently associated with some subsets of acute mixed lineage leukaemia (especially ALL with myelomonocytic features), chromosome studies in adult patients have only been reported occasionally in patients with heterogeneous cytologic features (Antonio *et al.*, 1992). Deletion of all or part of chromosomes 5 and 7 or the presence of complex changes confers an unfavorable prognosis. Other findings (e.g., normal karyotype, +8, 11q23) generally confer an intermediate prognosis (Kenneth *et al.*, 2010).

Acute myeloid leukaemia with Normal Karyotype

Acute myeloid leukaemia with normal karyotype is also referred to cytogenetically normal AML (CN-AML). AML patients with a normal karyotype who present between the ages of 16 and 60 years carry an intermediate prognosis. However, cytogenetically normal AML is highly heterogeneous at the molecular level, both mutations and over-expression of single gene have been identified, and their complex interactions are frequently refined to provide more accurate risk stratification. Within the normal cytogenetic category, 45% to 62% of patients with AML have NPM 1 (Nucleophosmin 1) mutations (localized on chromosome 5, band q35), 23% to 33% show FLT3 (*fms-like tyrosine kinase 3*) mutation localized on chromosome 13, band q12, 5% to 10% have MLL (mixed-lineage leukemia) tandem duplications (localized on chromosome 11, band q23), and 8% to 15% have CEBPA (**CCAAT Enhancer Binding Protein**) mutations (localized on chromosome 19, band q13.1). The prognosis of patients with normal karyotype differs in the presence of each of these mutations. In childhood AML with normal cytogenetics, NPM 1 mutations are relatively uncommon, occurring at a frequency of 8%. Currently, a newly diagnosed patient with AML and a normal karyotype should be checked for more common mutations such as FLT3, NPM 1, CEBPA, RUNX1, MLL-PTD, or EVI 1, which may have prognostic and possibly therapeutic implications (Ronald *et al.*, 2013).

Many studies have demonstrated that a mutation of the *FLT3* (*FMS-like tyrosine kinase 3*) gene is one of the most important prognostic factors in AML, and is associated with an adverse clinical outcome (Nurul *et al.*, 2011).

Another mutation that cannot be detected by conventional cytogenetics is internal tandem duplications (ITD). Internal tandem duplications have been detected with the highest incidence in patients with acute promyelocytic leukaemia (Nurul *et al.*, 2011).

The discovery of the driving oncogenic events such as gene mutations or other aberrations in

this subtype of AML is especially needed to find targets for the development of novel therapies and to determine prognostic factors for this group of patients. Despite having a normal karyotype, CN-AML is a very heterogeneous group of AMLs. This is reflected by the different prognostically relevant gene mutations that have been discovered in adult CN-AML recent years (Zwaan *et al.*, 2003).

Since there are some cases of AML with normal karyotype detected by conventional cytogenetics analysis, it was also documented that the outcome of normal cytogenetic AML patients varies. It is therefore, essential for the researchers to identify other genes involved in pathogenesis of normal cytogenetic AML using advanced techniques such as Array comparative genomic hybridization (array CGH), Nano string technology and sequencing methods.

Cytogenetics diagnoses of AML

Cytogenetics has become a compulsory aspect in the diagnoses of patient suspected of having AML. Because research has shown that approximately 55% of adults having AML has chromosomal abnormality which either falls on the WHO category: "AML with recurrent genetic abnormalities" and "AML with myelodysplasia-related features" (Mrozek et al., 2004).

Conventional karyotyping is the classical way of investigating chromosomal aberrations present in a leukaemic cell. The technique is based on the staining of the chromosomes with a dye after arresting the cell during cell division (metaphase) by colchicine. The resulting karyotype, i.e. the number and appearance of the set of chromosomes in one cell, is depicted in a karyogram. At least 20 metaphases are required for a reliable result, although fewer metaphases are also accepted in case of abnormal clone or clones (Mrozek *et al.*, 2004).

The following are some of the cytogenetic abnormalities encountered in AML with their genetic consequences due to chromosomal translocation.

Cytogenetic abnormalities regardless of blast count

- i. AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- ii. AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFβ-MYH11
- iii. APL with t(15;17)(q24.1;q21.1); PML-RARA.

t(8;21) translocation in AML

This balanced translocation t(8;21)(q22;q22); RUNX1-RUNX1T1 has been reported in adult patients that have been newly diagnosed with AML. It is also a very common disorder among children diagnosed with AML (Grimwade *et al.*, 2010). It results from fusion between RUNX1 or core binding factor alpha 2 gene on chromosome 21 with the *Runt Related Transcription Factor 1* (RUNX1T1) gene on chromosome 8 to form RUNX1-RUNX1T1 chimeric product that regulate the transcription of number genes that are important in hematopoietic stem and progenitor cell growth, differentiation and function. This type of translocation is also seen in AML-M2 and AML-M4. Adults with t(8;21) type of AML have a favorable prognosis in adults but in children the prognosis are poor (Kozu *et al.*, 1993).

t(15;17) translocation in APL

t(15;17) balanced translocation is seen in APL even though they are occasionally found in chronic myeloid leukemia (CML). The translocation results from the fusion of promyelocytic leukaemia (PML) gene on chromosome 15 with retinoic acid receptor alpha (RARA) gene on chromosome 17 resulting in formation of PML-RARA fusion protein. The fusion of this genes leads to the persistent transcription repression thereby preventing differentiation of promyelocytes. This kind of mutation has an excellent prognosis as it can be reversed by giving a pharmacological dose of all trans retinoic acid, thus leading to terminal differentiation of promyelocytes (Melnick and Licht, 1999). APL is a unique clinic-pathological entity characterized by the infiltration of the bone marrow by promyelocytes in association with clinical or laboratory evidence of disseminated intravascular coagulation (DIC) and fibrinolysis, which may worsen during the

initial cytolytic response to chemotherapy. A characteristic folded, kidney-shaped, or bilobed nucleus is invariably found in some of the promyelocytes; coarse azurophilic granules and multiple Auer rods are also common (Bitter *et al.*, 1987). PML/RARA gene is routinely identified within 24 hours by PCR methods, allowing confirmation of the diagnosis of APL.

Inv(16) and t(16;16)

The abnormality of chromosome 16 can either be AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); Core binding factor beta – Myosin heavy chain 11 (CBFβ-MYH11) or AML with other abnormalities of chromosome 16. The former has good prognoses while the later do not. In AML with inv(16), the morphological examination of the bone marrow demonstrates monocytes and granulocytes with abnormal eosinophils and thus it was previously called AML-M4 and with abnormal eosinophils is called AML-M4Eo.

The inversion breakpoint at 16q22 occurs near the end of the coding region of the CBFβ gene, which encodes one subunit of the heterodimeric RUNX1/CBFβ transcription factor. This transcription factor binds directly to an enhancer core motif that is present in the transcriptional regulatory regions of a number of genes that are critical to myeloid cell growth, differentiation, and function. MYH11 gene is interrupted by the breakpoint on 16p. A fusion protein is produced containing the 5' region of CBFβ (165 of 182 amino acids), including the domain that heterodimerizes with RUNX1, fused to the 3' portion of MYH11 (Shurtleff *et al.*, 1995; Liu *et al.*, 1993). The CBFβ/MYH11 fusion protein appears to act by disrupting the function of the RUNX1/CBFβ transcription factor, resulting in the repression of transcription.

Cytogenetic abnormalities which require at least 20% of myeloid blast for diagnoses

- i. AML with t(9;11)(p22;q23); KMT2A-MLLT3 or rearrangement of 11q23
- ii. AML with t(6;9)(p23;q34); DEK-NUP214
- iii. AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-MECOM
- iv. AML (Megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1

AML with 11q23 rearrangement

Chromosomal abnormalities of 11q23 and the rearrangement in mix lineage leukemia (MLL) gene are very important marker in the prognostic stratification of AML and MDS (Boris and Lisa, 2015). 11q23 translocation is one of the most common cytogenetic abnormality that was first reported in 1970 by Vande Berghe *et al.* (1979) where they describe it in cases of ALL with t(4;11)(q21;q23) (Ibrahim *et al.*, 2000). And it was later reported in other malignant neoplasm with chromosomal abnormalities. Abnormalities of 11q23 was also reported by Bower *et al.* (1994) to be more common in leukaemic cell that can differentiate into myelomonocytic cells and thus rearrangement in 11q23 was noted to be more common in patient with acute monoblastic leukaemia. (AML M5) and were associated with poorly differentiated monoblasts (AML-M5a) (Berger *et al.*, 1982).

The 11q23 region has been shown to be involved in non-random chromosomal translocations with a number of different partner chromosomes in acute leukemia. These include 1p32,4q21, and 19p13 in ALL, and 1q21,2p21,6q27, 9p22, 10p11, 17q25, and 19p13 in AML, especially monoblastic and myelomonocytic subtypes (M4 and M5). The most common translocation in infant leukaemia is t(4;11)(q21;q23), which is usually associated with a lymphoblastic or a mixed lymphoblastic/monocytic phenotype (Alejandra *et al.*, 2015). The translocation of 11q23 involves the rearrangement of the MLL gene also called KMT2A (lysine k specific methyl transferase 2A). This Translocation can be detected by Southern blotting analysis using a small Cdna probe containing these exons (Thirman *et al.*, 1993). Translocations of KMT2A result in the formation of a chimeric gene on the derivative 11 chromosome, consisting of the 5' region of KMT2A and the 3' region of the partner gene from the other chromosome, with subsequent expression of fusion mRNAs. The most common translocation involves KMT2A and the MLLT3 (AF9) gene at 9p22 (Swerdlow *et al.*, 2008). A key feature of KMT2A fusion proteins is their ability to efficiently transform hematopoietic cells into leukemia stem cells and therefore it has been presumed that altered KMT2A function

contributes to leukemogenesis. In general, leukaemia patients with 11q23/KMT2A rearrangements, have a very disappointing prognosis regardless of whether it is of myeloid or lymphoid lineage involvement, particularly infant ALL patients with the t(4;11). In contrast, patients with t(9;11)(p22;q23) tend to have an intermediate response to standard therapy (Biswas *et al.*, 2011).

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

This type of leukaemia typically presents with basophilia, pancytopenia and dysplasia. The pathological features of AML with the t(6;9) include basophilia, single or multilineage dysplasia in adult patients, variable FAB morphology, immunophenotyping shows a CD13+, CD33+, CD38+, CD45+, and HLA-DR+, and a high incidence (71%) of fms like tyrosine kinase 3 (FLT3 internal tandem duplications). The translocation results in the juxtaposition of DEK on chromosome 6 with NUP214 (also known as CAN) on chromosome 9. This results in the creation of a nucleoporin fusion protein that acts as a transcription factor and also alters nuclear transport. Patients with the t(6;9)(p23;q34) typically have a poor prognosis with standard therapy (Chi *et al.*, 2008).

t(3;3) and inv(3) in AML with thrombocytosis

The t(3;3) and inv(3) account for approximately 1 percent of AML cases. This abnormality is seen in de novo AML and in therapy-related MDS/AML. Cytogenetic abnormalities of 3q are associated with thrombocytosis in the peripheral blood and increased atypical megakaryocytes in the bone marrow of patients with AML (Pintado *et al.*, 1985). The specific cytogenetic abnormalities of 3q are 3q21 and 3q26.2 simultaneously, and they include the inv(3)(q21q26.2), t(3;3)(q21;q26.2), and the ins(5;3)(q14;q21q26.2) (insertion of chromosomal material from 3q into 5q). The t(3;3) and inv(3) abnormalities seen in AML result in the activation of the MECOM (EVII) gene, located at 3q26.2. The chromosomal rearrangements that lead to this activation are either 5' of the gene in the t(3;3) or 3' of the gene in the inv(3) (Langabeer *et al.*, 2001).

MECOM encodes a zinc-finger transcription factor that interacts with a number of transcriptional and epigenetic regulators (CREBBP, CTBP, HDAC, KAT2B [P/CAF], SMAD3, GATA1, GATA2, DNMT3A, and DNMT3B), and mediates chromatin modifications and DNA hypermethylation. Depending on its binding partners, MECOM can act as a transcriptional activator to promote the proliferation of hematopoietic stem cells (e.g., when bound to GATA2) or as a transcriptional repressor inhibiting erythroid differentiation (e.g., when bound to GATA1). Abnormal expression of EVI1 (MECON) has also been detected in patients with myeloid leukemia and a normal karyotype, suggesting that inappropriate activation of this gene occurs through various mechanisms, and may confer an adverse prognosis (Russell *et al.*, 1994).

AML (Megakaryoblastic) with t (1; 22) (p13; q13); RBM15-MKL1

AML (Megakaryoblastic) with t (1; 22) (p13; q13); RBM15-MKL1 is a very rare abnormality that is seen in infants but not in those with Down syndrome. It is characterized by the present of megakaryoblast. This translocation involves the RNA-binding motif protein-15 (RBM15, also known as OTT) and a DNA binding motif protein known as megakaryocyte leukemia-1 (MKL1, also known as MAL). MKL1 is involved in the normal production of platelets (Cheng *et al.*, 2009). The role of the resultant chimeric protein

in leukemogenesis is poorly understood and the prognostic significance of t (1; 22) (p13; q13) with modern therapy is also unclear.

Prognostic importance of differential karyotypes in acute myeloid leukemia

Based on several large studies, a cytogenetic risk stratification system has been proposed for AML that categorizes specific karyotypic abnormalities as favorable, intermediate, or unfavorable.

Patients with a normal karyotype constitute about 30% to 35% of patients. Karyotypes associated with a better than average response to standard therapy, includes inv (16), t(16;16), t(8;21), and t(15;17), and comprise of <30% of patients or <20% if older patients are included. Patients with a normal karyotype, and have trisomy 8 or trisomy 21 has an intermediate prognosis. Patients with most other chromosomal abnormalities including -5, del(5q), -7 or del(7q), inv(3p) most abnormalities of 11q, 20q, 21q, 17p, del(9q), t(9,22) or complex karyotypes, have poor prognoses. However, some patients with a normal karyotype when analyzed with standard methodology do have a t (15; 17), t (8;21), inv (16), or 11q abnormality that is detectable if PCR techniques are used. It should be noted that each group varies according to response to treatment (Bullinger *et al.*, 2004).

Table 1: Prognostic importance of differential karyotypes in acute myeloid leukemia

Prognosis of myeloid acute leukemia based on cytogenetic results	Cytogenetic findings
Favorable prognosis	
Balanced structural rearrangements	t (15;17) (q22; q 12-21), t (8; 21) (q22; q22), inv (16) (p13q22)/t (16;16) (p13; q22)
Intermediate prognosis (normal karyotype)	
Balanced structural rearrangements	t (9 ;11) (p22; q23)
Unbalanced structural rearrangements	del (7q), del (9q), del (11q), del (20q)
Numerical aberrations	-Y, +8, +11, +13, +21
Poor prognosis	
Balanced structural rearrangements	Inv (3) (q21q26)/t (3 ; 3) (q21 ;26), t (6 ; 9) (p23; q34), t (6 ;11) (q23; p13,1)
Unbalanced structural rearrangements	del (5q)
Numerical aberrations	-5, -7

Source: **Bullinger *et al.*, 2004.**

Molecular diagnoses of AML

Patients suspected of having AML, molecular diagnoses should be done on their blood or bone marrow specimen. DNA or RNA is extracted and then the viable cells are stored, but RNA extraction is better for molecular screening of fusion genes. Some of the somatically acquired mutations that have been identified in several genes are:

1. Nucleophosmin 1 (NPM1) gene
2. Fms-like Tyrosine Kinase 3 *FLT3* gene
3. CCAAT Enhancer Binding Protein (*CEBPA*) gene
4. Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, *Drosophila*) (*MLL*) gene
5. Runt-related transcription factor 1 (*RUNX1*) gene
6. Tet oncogene family member 2 (*TET2*) gene
7. Isocitrate dehydrogenase 1 (NADP+), soluble (*IDH1*) gene.
8. Neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*) gene
9. Wilms tumor 1 (*WT1*) gene
10. V-kit Hardy-Zuckerman 4 feline sarcomaviral oncogene homolog (*KIT*) gene

The frequencies of these gene mutations vary among cytogenetic groups (Dohner *et al.*, 2010).

Conclusion

Cytogenetics and molecular profiles are importance aspects to be considered in the diagnosis and prognosis of acute myeloid leukaemias. Nowadays, no diagnosis is of optimal important without cytogenetics and molecular analysis because they determine the overall survival of AML patients in many cases.

Recommendations

1. The discovery of the driving oncogenic events such as gene mutations or other aberrations in CN-AML is needed which will help to find targets for the development of novel therapies and to determine prognostic factors for this group of patients.
2. With the advancement in molecular technology, it is therefore, possible for the researchers to identify the genes involve in

pathogenesis of normal cytogenetic AML using advanced techniques such as Array comparative genomic hybridization (array CGH), Nano string technology and sequencing methods.

References

- Alejandra, S.P., Clara, B., Cristina, P., Pamela, A., Ronald, W.S., Rolf, M., and Pablo, M. (2015). Revisiting the biology of infant t(4;11)/MLL-AF4⁺ B-cell acute lymphoblastic leukemia. *;* 126(25): 2676–2685.
- Antonio, C., Jean-Louis, M., Augustin, F., Luc, V. H., Andre, B., Michel, S., Paola, D.C., Elisabeth, V., Jean-Jacques, C., Massimo, N., Nadia, P., Gianluigi, C., and Herman, V.D.B. (1992). Correlation of cytogenetic patterns and clinicobiological features in adult acute myeloid leukaemia expressing lymphoid markers, *Blood*; 79(3):720.
- Berger, R., Bernheim, A., Sigaux, f. (1982). Acute monocytic leukemia chromosome studies. *Leukaemia Research*; 6:17.
- Biswas, D., Milne, T.A. and Basrur, V. (2011). Function of leukemogenic mixed lineage leukemia 1 (MLL) fusion proteins through distinct partner protein complexes. *Proceeding of the National Academy of Science USA*; 108:15751.
- Bitter, M.A., Le Beau, M.M. and Rowley, J.D. (1987) Associations between morphology, karyotype, and clinical features in myeloid leukemias. *Humam Pathology*; 18:211.
- Boris, G. and Lisa, W. (2015). Leukemogenic rearrangements at the mixed lineage leukemia gene (*MLL*)-multiple rather than a single mechanism. *Cell and Developmental Biology*; 3(41):1-13.
- Bower, M., Parry, P. and Carter, M. (1994). Prevalence and clinical correlations of MLL gene rearrangement in AML-M4/M5. *Blood*; 84:3766-3768.
- Bullinger, L., Dohner, K. and Bair, E. (2004). Use of gene expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. *New England Journal of Medicine*; 350(16):1605-1616.
- Cheng, E.C., Luo, Q. and Bruscia, E.M. (2009). Role for MKL1 in megakaryocytic maturation. *Blood*; 113:2826.

- Chi, Y., Lindgren, V., Quigley, S. and Gaitonde, S. (2008). Acute myelogenous leukemia with t(6;9)(p23;q34) and marrow basophilia: An overview. *Archives of Pathology Laboratory Medicine*; 132: 1835-1837.
- Deschler, B. and Lubbert, M, (2006). Acute myeloid leukemia: epidemiology and etiology. *Cancer Journal*; 105(9):127-365.
- Dohner, H., Estey, E.H. and Amadori, S. (2010). Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European Leukemia- Net. *Blood*; 115: 453-474.
- Frohling, S., Scholl, C., and Gilliland, D.G. (2005). Genetics of myeloid malignancies: Pathogenetic and clinical implications. *Journal of Clinical Oncology*; 23:6285-6295.
- Grimwade, D., Hills, R.K., Moorman, A.V. (2010). Refinement of cytogenetic Classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the Kingdom Medical Research Council trials. *Blood*; 116:354.
- Hahn, C, (2015). Genomic aberrations of myeloproliferative and myelodysplastic/myeloproliferative neoplasms in chronic phase and during disease progression. *International Journal of Laboratory Haematology*; 37:181-189.
- Ibrahim, S., Elihu H. E., Sherry P., Armand G., Michael K., Susan O'B., Hagop M. K., and Maher A., (2000). 11q23 Abnormalities in Patients with Acute Myelogenous Leukemia and Myelodysplastic Syndrome as Detected by Molecular and Cytogenetic Analyses. *American Journal of Clinical Pathology*; 114:793-797.
- Kenneth, K., Marshall, A.L., Ernest, B., Thomas, J.K., Uri, S., and Josef, T.P. (2010). *Williams Haematology*. 8th ed. New York, US: McGraw-Hill companies:1277, 1282, 1284.
- Kozu, T., Miyoshi, H. and Shimizu, K. (1993). Junctions of the AML1/MTG8 (ETO) fusion Are constant in t (8; 21) acute myeloid leukaemia detected by reverse transcription polymerase chain reaction. *Blood*; 82:1270.
- Langabeer, S.E., Rogers, J.R. and Harrison, G.L. (2001). EVI1 expression in acute myeloid leukaemia. *British Journal of Haematology*; 112:208.
- Liu, P., Tarlé, S.A. and Hajra, A. (1993). Fusion between transcriptionfactor CBF beta/PEBP2 beta and a myosin heavy chain in acute myeloid leukemia. *Science*; 261:1041.
- Melnick, A., Licht, J.D. (1999). Deconstructing a disease: RARalpha, its fusion partners, and their roles in the pathogenesis of acute promyelocytic leukemia. *Blood*; 93:3167.
- Mro'zek, K., Heerema, N.A. and Bloomfield, C.D. (2004). Cytogenetics in acute leukemia. *Blood Review*; 18(2):115-136.
- Nelson, H. (2008) Leukaemia: genetics and prognostic factors. *Journal of Pediatrics*; 84(4):52-57.
- Nurul, A. F. A., Rosline, H., Abu, D. A., Ang, C. Y., Selamah, G., Sarina, S., Abdul Aziz, B. and Azlan, H. (2011). Low incidence of *FLT3* gene mutation among Malay patients with Acute Myeloid Leukaemia: A preliminary study. *Asia-Pacific Journal of Molecular Biology and Biotechnology*; 19 (2): 57-62.
- Piller, G. (2001). Leukaemia - A brief historical review from ancient times to 1950. *British Journal of Haematology*; 112:282-292.
- Pintado, T., Ferro, M.T. and San, Román. C. (1985). Clinical correlations of the 3q21; q26 cytogenetic anomaly. A leukemic ormyelodysplastic syndrome with preserved or increased platelet production and lack of response to cytotoxic drug therapy. *Cancer*; 55:535.
- Redaelli, A., Stephens, J. M., Laskin, B. L., Pashos, C. L. and Botteman, M. F, (2003). The burden and outcomes associated with four leukemias: AML, ALL, CLL and CML. Expert Review of Anticancer Therapy; 3:311-329.
- Ronald, H., Edward, J.B., Leslie, E.S., Helen, H., Jeffrey, W. and John, A. (2013). *Hematology Basic Principle and Practice*. Vol. 1; 6th ed. Ontario, Canada: Saunders, an Imprint of Elsevier Inc.: 749-750.
- Rui, Z. Ji-Yun, L. Xianfu, W. Weihong, X. Xiaoxia, H. Xianglan, L. Yimeng, N. Rurong, T. Shibo, L. and Yan, L. (2014).

- Identification of Novel Genomic Aberrations in AML-M5 in a Level of Array CGH. *PLoS One*; 9(4): e87637.
- Russel, M., List, A. and Greenberg, P. (1994). Expression of EVI1 in myelodysplastic syndromes and other hematologic malignancies without 3q26 translocations. *Blood*; 84:1243.
- Shurtleff, S.A., Meyers, S. and Hiebert, S.W. (1995). Heterogeneity in CBF beta/MYH11 fusion messages encoded by the inv (16) (p13q22) and the t(16;16) (p13;q22) in acute myelogenous leukaemia. *Blood*; 85:3695.
- Swerdlow, S.H., Campo, E. and Harris, N.L. (2008). World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues. *IARC Press*, Lyon.
- Thirman, M.J., Gill, H.J. and Burnett, R.C. (1993). Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. *New England Journal of Medicine*; 29:909.
- Van den Berghe H, David G, Broeckaert-Van Orshoven A. (1979). A new chromosome anomaly in acute lymphoblastic leukaemia (ALL). *Human Genetics*; 46:173-180.
- Wertheim, G.B., Hexner, E. and Bagg, A. (2012). Molecular-based classification of acute myeloid leukaemia and its role in directing rational therapy: personalized medicine for profoundly promiscuous proliferations. *Molecular Diagnostic Therapy*; 16 (6):357-369.
- Zwaan C.M., Meshinchi, S. and Radich, J.P. (2003). FLT3 internal tandem duplication in 234 children with acute myeloid leukemia: prognostic significance and relation to cellular drug resistance. *Blood*; 102:2387-2394.

Citation: Ajayi O.I., and Garba, N. Differential Molecular Diagnosis in Acute Myeloid Leukaemia: A Review. *Sokoto Journal of Medical Laboratory Science*; 3(3): 71 - 79.

Copyright. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.