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Molecular Genetic Tests for The Diagnosis of Haematological Malignancies: A Review

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Abstract

Haematologic malignancy are blood and bone marrow disorders that account for about 9% of all cancers and are estimated to be the fourth most frequently diagnosed cancer in developed countries. They are made up of tumors of lymphoid, myeloid, histiocytic, and dendritic cell lineages with each disease defined based on the assemblage of its morphologic, clinical, biological features and the cell of origin. Haematologic malignancies can be grouped as leukaemia, lymphoma and myeloproliferative disorders depending on the cell lineage. Molecular genetic testing can be used to diagnose haematological malignancies in order to establish or confirm new diagnosis, determine disease stage, monitor the efficacy of therapy, detect minimal residual disease, and to predict outcome of treatment. This review involved the search of literatures on PubMed, Medline and Advanced Google search using the keywords described in this text with related articles pooled and relevant information extracted and properly referenced. The genetic tests will detect individual mutations in oncogenes; chromosomal translocations, deletions, and duplications; and clonality in lymphoma and leukaemia. Apart from cytogenetic which has its own disadvantages based on acquisition of a reliable bone marrow sample the more reliable molecular techniques developed include fluorescence in situ hybridization (FISH); Polymerase Chain Reaction (PCR); Reverse-Transcription Polymerase Chain Reaction (RT-PCR); Real-Time Quantitative Polymerase Chain Reaction (Q-PCR); DNA Sequencing; Aptamer-Conjugated Nanoparticles for Selective detection of leukaemic cells; and Next Generation Sequencing. The emergence of different molecular based genetic tests

which are highly sensitive and specific have made it easier for prompt and accurate diagnosis of different haematological diseases with next generation sequencing being another great tool for identifying disease associated genetic changes in a genome-wide fashion.

Keywords: Haematological malignancies, molecular diagnosis, genetic tests.

Introduction

Haematological malignancies (also termed haematologic neoplasms) are a diverse group of blood and bone marrow diseases with a myriad of different presentations (Anne, 2009). They are comparatively common, accounting for around 9% of all cancers and estimated as the fourth most frequently diagnosed cancer in both men (after prostate, lung, and colorectum) and women (after breast, lung, and colorectum) in economically developed regions of the world (Maria *et al.*, 2011). Haematological neoplasms consist of tumors of lymphoid, myeloid, histiocytic, and dendritic cell lineages. Each disease is defined as a distinct entity based on a constellation of morphologic, clinical, and biological features and the cell of origin is the starting point of disease definition. They are characterized by uncontrolled proliferation of one or more blood cell lines which if not well managed or treated results to series of severe



conditions such as epistaxis, immunosuppression, infections, disseminated intravascular coagulopathy, anaemia and eventually death of the patient (Maria *et al.*, 2011).

Depending on the cell line involved, haematologic malignancies can be grouped as leukemia, lymphoma and myeloproliferative disorders. Leukaemia are classified into subtypes; chronic myeloid leukaemia; chronic lymphocytic leukaemia; acute lymphoblastic leukaemia and acute myeloblastic leukaemia (Swedlow *et al.*, 2008) while the lymphoma are classified as precursor lymphoid neoplasm consisting of B-lymphoblastic leukaemia/lymphoma and B-lymphoblastic leukaemia/lymphoma; Mature B-cell neoplasm and Hodgkin's lymphoma while the Myeloproliferative neoplasms (MPN) are classified as polycythemia vera, essential thrombocythemia, primary myelofibrosis and chronic myeloid leukemia, the less common types of myeloproliferative disorders considered by WHO includes systemic mastocytosis, chronic eosinophilic leukaemia, and chronic neutrophilic leukaemia.

Some lymphomas and leukemias can be identified by routine morphologic approaches. However, for many diseases, knowledge of the immunophenotype and molecular genetics/cytogenetics plays an important role in the differential diagnosis. The sites of presentation and involvement are an important clue to underlying biological distinctions (Anne, 2009).

Methodology

This review involved search of literatures on PubMed, Medline and Advanced Google search using the keywords described in this text with related articles pooled and relevant information extracted and properly referenced. Textbooks and journals from the University of Benin and Afe Babalola University libraries were also searched, used and well referenced for the purpose of this review.

Findings

Leukaemia

Leukaemia are group of disorders affecting haemopoietic stem cell compartment, characterised by uncontrolled proliferation and accumulation of malignant white cells in the bone marrow and peripheral blood (Mathers *et al.*, 2001). Leukaemia is the second most common form of cancer in infants, however over 90% of all leukaemias are diagnosed in adults (Mathers *et al.*, 2001).

Different types of leukaemia have been recognized in human population. Clinically and pathologically, leukaemia is subdivided into acute and chronic forms. Acute leukaemia is characterized by a rapid increase in the number of immature blood cells crowding the bone marrow which prevents the bone marrow from producing normal healthy blood cells. Chronic leukaemia on the other hand is characterized by the excessive buildup of relatively mature, but still abnormal white blood cells typically taking months or years to progress. The cells are produced at a much higher rate than normal, resulting in many abnormal white blood cells (Jameson *et al.*, 2005). Additionally, leukaemias are subdivided to lymphocytic and myelogenous leukaemias, depending on the cell lineage affected. Combining these two classifications provides a total of the four main categories; acute lymphoblastic leukaemia (ALL), acute myelogenous leukaemia (AML), chronic lymphocytic leukaemia (CLL), chronic myelogenous leukaemia (CML) (Swedlow *et al.*, 2008).

Leukaemia like other cancers, results from mutations in the DNA. Certain mutations can trigger leukaemia by activating oncogenes or deactivating tumour suppressor genes, and thereby disrupting the regulation of cell death, differentiation or division (Ross *et al.*, 2002). The known causes of leukaemia in adult are ionizing radiation, a few viruses such as human T-lymphotropic virus, some chemicals notably benzene, some alkylating chemotherapy agents for previous malignancies, some petrochemicals and hair dyes (Wiernik *et al.*, 2001), and use of tobacco (Robinette *et al.*, 2001). In addition,

people with genetic issues such as chromosomal abnormalities or certain genetic conditions have a greater risk of leukaemia. Diet has very limited or no effect, although eating more vegetables may confer protective benefit (Wiernik *et al.*, 2001).

The signs and symptoms of leukaemia include displacement of normal bone marrow activities, higher numbers of immature white blood cells (acute) resulting to low platelet count and anaemia, bruises, excessive bleeding, dysfunctional white cell, immunosuppression, frequent infection, fever, feeling sick, fatigue, hepatosplenomegaly, weight loss etc. In some leukaemia patients, a high white blood cell count is not visible during a regular blood count and this condition is called aleukemia (American Cancer Society, 2010). Complete blood count and bone marrow examination can be done for routine diagnosis of leukaemia (American Cancer Society, 2010).

Acute lymphoblastic leukaemia (ALL): This is a rapidly developing disease that is characterized by large numbers of immature lymphocytes (Jameson *et al.*, 2005). It is the most common type of leukaemia in young children. This disease also affects adults especially those above 65 years and older (Geoffrey *et al.*, 1985). The survival rate varies by age, 85% in children and 50% in adults.

Acute Myeloblastic Leukaemia (AML): This disease is characterized by production of large numbers of immature granulocyte myeloid cells (immature neutrophils -- the most common, monocytes, basophils, eosinophils, platelets or red blood cells) that replace other normal cells in the marrow. It affects people of all ages but more common in adult than children and more common in men than women. The 5-year survival rate is 40%. Subtypes include acute prolymphocytic leukemia, acute myeloblastic leukemia and acute megakaryoblastic leukemia (Colvin *et al.*, 2003).

Chronic Lymphocytic Leukaemia (CLL): This disease progresses more slowly and it affects adult over the age of 55 and sometimes

occur in younger adults but it almost never affects children. The 5-year survival rate is 75%. One subtype is B cell prolymphocytic leukemia which is a more aggressive disease (Cancer Stat Factsheet, 2012).

Chronic Myelogenous Leukaemia (CML): Chronic myelogenous leukaemia is found mainly in adults and rarely in children. It is an acquired condition that begins in an immature stem cell in the bone marrow when pieces from two chromosomes (9 and 22) break off and switch places (translocation). This results in an altered, fused gene (BCR/ABL) on chromosome 22 that produces a protein called tyrosine kinase that affects cell growth regulation. This leads to an overproduction of granulocytic white blood cells, many with the BCR/ABL translocation, and the presence of both mature and immature cells in the bloodstream. Within each of these four main categories, there are typically several subcategories e.g. precursor B acute lymphoblastic leukaemia, B-cell prolymphoblastic leukaemia, Hairy cell leukaemia, Burkitt's leukaemia etc (Jameson *et al.*, 2005).

Diagnosis of Leukaemia

Leukaemia can be diagnosed by the following techniques (Barbara, 2005);

- Full blood count and blood film which is essential in all patients to determine the increase in the granulocytes and lymphocytes, the cell morphology to detect the exact cell that is over populated.
- Bone marrow aspirate and biopsy is essential in all patients to detect a hypocellular or fibrotic marrow.
- Cytochemical stains is useful in diagnosis to differentiate the type of leukaemia especially if a leukaemia is not obviously myeloid.
- Immunophenotyping which could be performed by flow cytometric technique is also essential in all patients in whom the leukaemia is not obviously myeloid; it can provide means of monitoring for minimal residual disease.



- Cytogenetic analysis is essential in all patients and its best performed on a bone marrow aspirate.
- Molecular genetic analysis including fluorescence *in situ* hybridization (FISH) though selective is important in monitoring for minimal residual disease optimizing treatment (Barbara, 2005).

Lymphoma

Lymphomas are a heterogeneous group of malignant neoplasms of the immune system arising from cells of the lymphoid series, mononuclear phagocytes, and reticular cells which make up the lymphatic system (Aisenberg, 2000). Progress in understanding the biology of lymphomas has led to the various classification systems and improvements in treatment modalities. The WHO classification which is a modification of Revised European American Classification of Lymphoma (REAL) Classification published in 2001, updated in 2008 grouped lymphomas by cell type (i.e. the normal cell type that most resembles the tumor) and defining phenotypic, molecular or cytogenetic characteristics into three large groups: The B cell, T cell and natural killer cell tumors (Maria *et al.*, 2011). This group is expatiated as the precursor lymphoid neoplasm, Mature B-cell neoplasm and Hodgkin lymphoma. The precursor lymphoid neoplasm consist of the B-lymphoblastic leukaemia/lymphoma and B-lymphoblastic leukaemia/lymphoma; the mature B-cell neoplasm is made up of the multiple myeloma and the following diffuse large B-cell lymphoma, Primary central nervous system lymphoma, Primary mediastinal B-cell lymphoma, Burkitt lymphoma/leukemia, Follicular lymphoma, Chronic lymphocytic leukemia/small lymphocytic lymphoma, B-cell prolymphocytic leukemia, Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia, Mantle cell lymphoma, Marginal zone lymphomas, Post-transplant lymphoproliferative disorders, HIV-associated lymphomas, Primary effusion lymphoma, Intravascular large B-cell lymphoma, Primary cutaneous B-cell and lymphoma Hairy cell leukemia (Maria *et al.*, 2011).

Myeloproliferative Neoplasm

The myeloproliferative disorders comprise several clonal haematologic diseases that are thought to arise from a transformation in a haematopoietic stem cell. The main clinical features of these diseases are the overproduction of mature, functional blood cells and a long clinical course (Goldman, 2003). MPN are classified based on the recognition of mutations/rearrangements in protein-coding genes with tyrosine kinase activity, such as *BCR-ABL* (break point cluster region –Abelson murine leukaemia) mutation in chronic myeloid leukemia [CML]), *JAK2* (Janus Kinase) in polycythemia vera [PV], primary myelofibrosis [PMF], essential thrombocythemia [ET]), and *KIT* (tyrosine protein kinase) (mastocytosis) (Johanson *et al.*, 2004). The myeloid and lymphoid diseases concomitant with *PDGFRA* (platelet-derived growth factor receptor A), *PDGFRB*, and *FGFR1* (fibroblast growth factor receptor1) rearrangements was important to define new disease groups and change previous diagnostic criteria. Less common conditions that are also considered myeloproliferative disorders under the classification used by the World Health Organization include systemic mastocytosis, chronic eosinophilic leukemia, chronic myelomonocytic leukemia, and chronic neutrophilic leukemia (Pierre *et al.*, 2001).

The cardinal features of the CML, PV, ET and PMF are an increased granulocyte, red-cell mass in polycythemia vera, a high platelet count in essential thrombocythemia, and bone marrow fibrosis in idiopathic myelofibrosis. These disorders share many characteristics, including marrow hypercellularity, a propensity to thrombosis and haemorrhage, and a risk of leukaemic transformation in the long term. The annual incidences of both polycythemia vera and essential thrombocythemia are 1 to 3 cases per 100,000 population; myelofibrosis is less common (Johanson, 2004).

Molecular Genetic Tests in Haematological Malignancies

Molecular genetic testing in haematological malignancies is used to establish or confirm

new diagnosis, determine disease stage, monitor the efficacy of therapy, detect minimal residual disease, and to predict outcome (Daniel, 2004). As discovery is progressing, molecular testing can be used to select therapies based on disease biology and patient genetics. Molecular genetic testing can be applied in haematologic malignancy in detecting mutations based on 3 broad categories which are;

1). Detection of individual mutations in oncogenes which detects the presence of mutations in the gene c-kit in mast cell tumors (Morley,1998). C-kit is a receptor tyrosine kinase for stem cell factor, which stimulates mast cell growth. C-kit mutations is manifested as internal tandem duplications (ITD) in c-kit occurring in approximately half of the mast cell tumors analyzed and this results in the uncontrolled proliferation of mast cells (London *et al.*, 1999; Morley, 1998).

2). Detection of chromosomal translocations, deletions, and duplications: One common feature of haematopoietic neoplasia is the presence of translocations that combine 2 genes to create a single fusion gene and protein product. These new fusion genes in turn provide a growth advantage to the tumor cell, and are an essential feature of oncogenesis (Anne, 2009). The best studied of these fusion genes is the Philadelphia chromosome also called the BCR-ABL fusion gene found in more than 90% of all human chronic myeloid leukemias(CML) and occasionally ALL and AML (Wong *et al.*,2009). ABL is a tyrosine kinase that has myriad activities involved in cell growth and differentiation. It is encoded on human chromosome 9, and translocated to chromosome 22 in CML. The site of the translocation varies within breakpoint cluster region (BCR) gene, so that a new fusion gene, BCR-ABL, is formed. The new fusion protein formed allows for the constitutive activation of the ABL tyrosine kinase, which, in turn, promotes the development of CML. The BCR-ABL translocation can also be found, sporadically, in some acute B-cell leukemias (Wong *et al.*,2009).

3). Detection of clonality in lymphoma and leukemia by taking advantage of the unique antigen receptor genes of B- and T-cells: Any diagnostic test that demonstrates that a group of cells is derived from a single clone can be considered a clonality assay. Clonality assay is used refer to series of genetic tests that involves detection of the unique genes found in each individual B- or T-cell which are Immunoglobulin (Ig) genes in B-cells and T-cell receptor genes in T-cells. The portion of these genes that encodes the antigen-binding region is the portion that varies between cells, both in size and sequence. Once a B- or T-cell is mature and divides in response to antigenic stimulation, the Ig and T-cell receptor genes are passed on to the daughter cells (Blom, 2006 and Delves *et al.*,2000).

In response to invading pathogen, B- and T-cells are activated, undergo clonal expansion, and eventually die, leaving behind only a small number of residual memory cells. Conversely, when a cell becomes neoplastic, it is no longer subjected to growth controls, and can expand significantly more than the normal cells in immune response. Consequently, if it can be established that the majority of cells in a particular collection of lymphocytes have the same Ig or T-cell receptor gene, it is most likely that these cells are neoplastic rather than reactive (Sweldlow, 2003).

In diagnosis of haematological malignancies, cytogenetics is a less time-consuming assay for the detection of overall and specific chromosomal abnormalities, such as translocations, partial deletions and aneuploidies (Tohami *et al.* 2012; Schoch *et al.*,2002). However, bone marrow cells and at least 20 banded metaphases that are required for cytogenetics examination are difficult to obtain. Furthermore, other difficulties in cytogenetics include poor quality metaphases, small sample size and the fact that only large size alterations are revealed and these all contribute to its low sensitivity. In addition, the need for cell culture and metaphase analysis, which are both labor intensive and technically demanding, have also limited its application (Wan *et al.*,2012; Baccarini *et al.*,2009). Emergence of molecular-



cytogenetic techniques, such as fluorescence *in situ* hybridization (FISH), and other molecular technologies have overcome many of the challenges encountered with conventional diagnostic methods. The molecular genetic tests are described as follows;

Fluorescence in situ hybridization (FISH)

FISH was developed in early 80s and became one of the most sensitive assays for localization of specific nucleic acid sequences and detection of numerical chromosome abnormalities, structural chromosomal rearrangements and cryptic abnormalities (Bauren *et al.*, 1980). Bone marrow aspirate and peripheral blood smears can be used for FISH analysis (Tohami *et al.*, 2012; Wan *et al.*, 2012). FISH technique makes use of fluorescent probes which will hybridize only to complementary sequences and the resulting signals are scanned under a fluorescent microscope. The efficacy of specific FISH probes provides powerful signals and several targets can be visualized simultaneously by using probes labeled with different fluorescent colors. Varieties of fluorescent probes are commercially available and commonly used probes in clinical routine laboratory include centromeric enumeration probes (CEP), chromosome painting probes, dual-color dual fusion probes (D-FISH) and dual-color break-apart probes.

CEP is used for enumeration of chromosomes by hybridizing to the unique alpha (or beta) satellite repeats within the centromeric regions; Chromosome painting probes are used to highlight the entire chromosome of interest while D-FISH are used to detect chromosomal translocations such as the BCR-ABL gene in CML.

The development of D-FISH is significant for diagnosis and treatment monitoring of diseases. D-FISH probe detects cryptic rearrangement and has a low false-positive rate which is an added advantage of the technique. It employs large probes that span the upstream and downstream of the target breakpoints on both chromosomes, and each probe is labeled with a

different color, such as red and green. There are two red signals and two green signals in normal cells. In affected cells where translocations present, two fused red/green signals from the translocated chromosomes, together with a single red and green signal from the unaffected counterparts, would be observed during metaphases (Wan *et al.*, 2012). Cryptic insertion of the BCR to ABL gene at 9q34, t (9;22) variant in chronic myeloid leukemia (CML), and three-way translocation in CML and acute promyelocytic leukemia (APL) have been reported and can be identified by D-FISH (Bacarrini *et al.*, 2009). FISH is pivotal in the diagnosis and clinical management of haematological malignancies. Other applications include the demonstration of t(11;18)(q21;q21) in mucosa-associated lymphoid tissue (MALT) lymphoma (Tanaka *et al.*, 2010); the detection of clonal abnormalities (clonal assay) (deleted 5q, trisomy 8 and monosomy 7) in myelodysplastic syndromes (MDS) with minimal dysplasia; 11q23 abnormalities in acute myeloid leukemia (AML) (Schoch *et al.*, 2003); the demonstration of hyper- or hypo-diploidy and occult translocations in acute lymphoid leukemia (ALL), which is hard to grow in metaphase and is one of the difficulties of cytogenetic detections (Inaba *et al.*, 2013); myeloproliferative neoplasms (MPN) with eosinophilia and interstitial deletion on short arm of chromosome 4; chronic lymphocytic leukemia (CLL) with trisomy 12 and cryptic t(15;17) variant – ins(17;15) in APL (Campbell *et al.*, 2013).

The FISH technique detects cryptic BCR-ABL1 translocations in comparison to cytogenetic, it also detects all molecular variants in CML and minimal residual disease (MRD) at low leukaemic cell counts. Also, both metaphase and interphase cells can be used in FISH, whereas, the use of interphase cells minimizes laborious procedures of growing metaphase cells in cytogenetics. However, result interpretations can be challenging when there are too much signals overlapping (Tohami *et al.*, 2012; Testoni *et al.*, 2009). In clinical diagnosis, FISH is often used in parallel with

cytogenetics and/or other molecular techniques for diagnostics, confirmations and treatment monitoring.

Polymerase Chain Reaction (PCR)

PCR is molecular technique commonly used for detection of known mutations at the DNA level. PCR procedure involves 20-40 thermal cycles which is comprised of denaturation, annealing, and elongation in each cycle. Post-PCR procedures include gel electrophoresis, sequencing and melting curve analysis. Both bone marrow aspirate and peripheral blood can be used. Unlike FISH, PCR and its variants only detect breakpoints covered by designated specific primers. Examples of the application of PCR include *JAK2 V617F* mutations in polycythemia vera; *FLT3* (fms-like tyrosine kinase 3), *NPM1*, and *CEBPA* mutations in acute myeloid leukemia with normal cytogenetics (Zhang *et al.*, 2013); and gene rearrangements such as Ig heavy chain gene rearrangement for B-cell malignancies and T-cell receptor gamma chain gene rearrangement for T-cell malignancies (Evan *et al.*, 2007) as a clonal assay. There are various derivatives of PCR technology which are widely used in hematological malignancies testing and diagnosis. They include; RT-PCR, Q-PCR, multiplex PCR.

Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

RT-PCR is a molecular technique commonly used in routine laboratory to examine target gene expression that is significant to the pathogenesis of haematological malignancies. RT-PCR procedure is comprised of complementary DNA (cDNA) synthesis using target RNA as a template and cDNA PCR amplification. One-step RT-PCR, where the entire reaction occurs in a single tube, is widely used currently (Lam *et al.*, 2013).

In diagnostic services, RT-PCR is very useful in identification of disease associated transcripts that are often crucial for making clinical decisions. For example, the reciprocal translocation of chromosome 9 and 22 (t9; 22) in CML has 4 combinations (e1a2, b2a2 or

b3a2, e19a2) that generate p190, p210, and p230 *BCR-ABL1* transcripts respectively. The p210 is commonly found in CML whereas the p190, which has almost identical constitutive tyrosine kinase activity as the p210, is more commonly seen in ALL (Inaba *et al.*, 2013; Quintas-Cardama *et al.*, 2009). RT-PCR can accurately distinguish the breakpoint in variant transcripts at the level of gene expression. Moreover, RT-PCR detects translocations such as the presence of *AP12-MALT1* transcripts that are indicative of MALT-lymphoma (Tanala *et al.*, 2010); *RUNX1-ETO* transcripts in AML; *PML-RAR α* transcripts in APL; and *ETV6-RUNX1* transcripts in ALL (Inaba *et al.*, 2013).

RT-PCR also detects gene fusion resulting from interstitial deletion such as *PDGFRA*-associated chronic eosinophilic leukemia (Loules *et al.*, 2009). The technique is pivotal in detection and monitoring of specific breakpoint, for example the *BCR-ABL1* in CML, however, is not sensitive enough for monitoring *BCR-ABL1* transcript level during treatment (Tohami *et al.*, 2009; Wang *et al.*, 2001). Comparatively, quantitative RT-PCR is suggested to have high sensitivity up to 0.001-0.0001% and is commonly used for treatment monitoring nowadays (Tohami *et al.*, 2009).

Despite the fact that RT-PCR is useful in diagnosis, it has its own disadvantage sometimes because of RNA degradation, leading to false-negative results; Cross-contamination is another issue leading to false-positive results in all PCR technologies (Shibata *et al.*, 2010).

Furthermore, multiplex RT-PCR, in which multiple pairs of primers are added in one PCR reaction, is used for simultaneous detection of multiple gene transcript targets. The protocol was established by Pallisgaard and Colleagues (1998) and it contained 8 parallel PCR reactions for simultaneous detection of 29 chromosomal aberrations in leukemia, for example, *BCR-ABL* (e1a2, b2a2, b3a2) chimeric gene for ALL and CML; *MLL/AF6* in AML and *NPM-RARA* in APL (Pallisgaard *et al.*, 1998).



Simplified multiplex RT-PCRs for common translocations in leukaemia is being employed in order to reduce labor costs and significantly improve turnaround time (Pakakasama *et al.*, 2018). In addition to CML cases, multiplex RT-PCR has greatly improved the detection of typical and atypical *BCR-ABL* fusion transcripts. Apart from the three predominant fusion transcripts of e1a2, e13a2 and e14a2, a number of atypical transcripts such as e1a3, e13a3, e14a3, e19a2, e6a2 and e8a2, can also be analysed simultaneously by multiplex RT-PCR. Multiplexing technology has enhanced the speed and reliability of fusion transcript detection by PCR.

Real-Time Quantitative Polymerase Chain Reaction (Q-PCR)

Quantitative Polymerase Chain Reaction (Q-PCR) and Quantitative Reverse Transcriptase Polymerase Chain Reaction (Q-RT-PCR) allow the quantification of DNA and RNA molecules, respectively (Lam *et al.*, 2013). Q-PCR makes use of fluorochrome-labeled sequence-specific probes such as adjacent hybridization probe and TaqMan probe to measure the quantity of target amplicons in Realtime. Besides, a known amount of target or adjacent sequence is coamplified in the PCR reaction. This acts both as an internal control and a reference standard to determine the amount of target molecules. For example, in CML, DNA/cDNA of *ABL* is co-amplified as an internal marker with the target DNA/cDNA of chimeric *BCR-ABL*. Commonly used TaqMan probes consist of a reporter at 5'-end and a quencher at 3'-end. It hybridizes to complementary target DNA and is cleaved by Taq polymerase, which has 5' to 3' exonuclease activity, during primer elongation and emits fluorescent signal. The quantity of target DNA, which is proportional to the fluorescent signals emitted, is determined with reference to a standard curve. The standard curve is a serial dilution of the internal control sequence with a known concentration (Tohami *et al.*, 2009; Hue-Roy *et al.*, 2008). The establishment of relative or absolute (standard curve) quantification provides a well presentation of data. Besides, international scale also allows inter-laboratory comparison (Garba *et al.*, 2003).

Among all commonly used molecular techniques, Q-PCR is the most sensitive and is routinely used for treatment monitoring and detection of MRD such as in CML and CLL (Golsberg *et al.*, 2013; Moore *et al.*, 2013). The quantification of *BCR-ABL* transcript is important for determining disease status and progression. In MRD, a complete remission requires less than 5% of leukaemic blasts found in bone marrow. In order to achieve the requirement, conventional cytogenetics requires analysis of a large number of metaphases. The costly and laborious procedure has limited its uses. Although FISH is comparatively easier for interpretation by counting 100-200 cells, it requires in-depth knowledge and technical expertise (Tohami *et al.*, 2012; Schoch *et al.*, 2002). Q-PCR is thereby suggested to be the most sensitive and fastest method for the detection of MRD with no post-PCR procedures required, thus dramatically reducing the risks of cross-contamination.

DNA Sequencing

DNA sequencing is a post-PCR analysis and a confirmation method for mutations. Applications include AML with *NPM1* exon 12 mutation, AML with mutated *CEBPA* (not common), *FLT3* exon 14 tandem duplication and other mutated genes – *KIT*, *MLL*, *WT1*, *NRAS*, *KRAS*; and enquiries on normal or specific karyotypes (Zhang *et al.*, 2013; Rossetti *et al.*, 2008).

Aptamer-Conjugated Nanoparticles for Selective Detection of Leukemic Cells

Diagnosis and taxonomy of acute leukemia's depend on integration of information from morphologic and cyto-chemical examination along with data obtained from immunophenotyping analysis using flow cytometry (FCM) and cytogenetic or molecular analysis of diagnostic genetic mutations associated with prognosis (Salem *et al.*, 2012). As antibodies used in FCM may not detect all the molecular events related with the growth of malignancy or other factors that may be useful for clinical management, the study of cell

surface proteins with nanotechnology may develop treatment strategies. Creating probes to identify surface proteins can be used to classify tumors depending on the molecular features of these cells rather than their tissues of origin (Sefah *et al.*, 2009). In this account, using molecular aptamers is a new strategy of identifying novel biomarkers that can be used to increase effectiveness of therapy and survival of leukaemia patients (Yang *et al.*, 2014). Aptamers are synthetic nucleic acid ligands that can be produced against many targets including proteins, drugs and amino acids (Mallikaratchy *et al.*, 2011). Compared with antibodies, aptamers have several advantages. To name a few, aptamers have a higher affinity and are easily synthesized with limited toxicity. In addition, aptamers can also fold into three-dimensional conformations with unique ability to bind biomolecular ligands (Lakhin *et al.*, 2013). Making use of these advantages, Herr *et al.*, (2006) have chosen an aptamer for acute leukaemic cells with a specific sequence. In the study, they created an assay using aptamer-conjugated nanoparticles for quick recognition of acute leukemic cells using high-affinity DNA aptamers for signal detection (Herr *et al.*, 2006). An oligonucleotide of 88 base pairs, which has the capability of binding to acute leukaemic cells, was attached to magnetic and fluorescent nanoparticles. The fluorescent nanoparticles then provided a method of detection, while the use of magnetic nanoparticles eliminated the need for centrifugation and helped the removal of unwanted particles (Herr *et al.*, 2006).

Next Generation Sequencing

Over the past decade, the cost of sequencing per base has been driven down significantly as a result of the emergence of novel technologies such as massively parallel sequencing, or so called next generation sequencing (NGS). The completion of the human genome project in 2003, the Encyclopedia of DNA Elements (ENCODE) project (Eckar *et al.*, 2012) and studies of various whole cancer genomes, whole exomes and transcriptomes have brought new insights in diagnostic, prognostic, preventive and personalized medicine. The three most commonly used NGS platforms are Illumina,

Life SOLID, and Roche 454. NGS technology has demonstrated effective, accurate and robust performance for sequence detection and enumeration (Eckar *et al.*, 2012).

In the era of NGS, whole cancer genome sequencing has revealed novel candidate genes that may contribute to leukaemic pathogenesis. In T-cell acute lymphoblastic leukemia (T-ALL), which is predominant in male, the X-linked *PHF6* gene was reported to be a tumor suppressor gene and the mutational loss of *PHF6* is significantly associated with its pathogenesis (Kohlman *et al.*, 2013; Van *et al.*, 2010). Moreover, the diagnosis of CN-AML has been difficult due to cryptic manifestation. NGS studies have revealed a number of driver mutations such as *NPM1* and *CEBPA* mutations in AML (Matelli *et al.*, 2013; Grossman *et al.*, 2001). Other newly discovered potential markers include *TP53* mutations in MDS [44] and *NOTCH1*, *SF3B1* and *BIRC3* mutations in CLL (Puente *et al.*, 2011; Rossi *et al.*, 2011).

More than a dozen myeloid-focused NGS-based panels are now available from commercial and academic laboratories (Kuo *et al.*, 2015). NGS could provide a diagnostic setting where multiple genetic markers and patient genomes are sequenced in a massively parallel manner. Godley *et al.* (2012) suggested having a diagnosis setting that includes Q-PCR, microarray analysis, and NGS for haematological malignancies. NGS technology has not only contributed to clinical and therapeutic research but also to clinical service and prenatal diagnosis. NGS may be introduced into diagnostic services in the foreseeable future.

Conclusion

Despite the use of conventional methods such as morphological analysis, special phenotypic tests and cytogenetic analysis for the diagnosis of haematological malignancies, the emergence of different molecular based genetic tests which are highly sensitive and specific have made it easier for prompt and accurate diagnosis of different haematological diseases. Next generation sequencing is another powerful tool for identifying disease associated genetic



changes in a genome-wide fashion, with improved turnaround time, decreasing costs, and an expanding knowledge of the therapeutic and prognostic significance of the detected variants, NGS-based panel testing is likely to play a major role in the management of patients with haematological malignancies in the coming decade. There is therefore no doubt that molecular diagnostics will take a significant role in routine diagnosis of haematological malignancies in the nearest future especially in the developing countries, Nigeria inclusive.

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