A role of FLT3 gene mutation in acute myeloid leukemia patients from Pakistan

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Abstract
Natural hematopoiesis has been described by the development of blood cells which in turn is regulated by need of the body. If these regulatory systems were disrupted, the production of these cells becomes excessive. The changes mostly occur in the hematopoietic stem cells (HSC) which normally go on to form blood cells, but defects in the hematopoietic stem cells cause an increase circulation of leukemic cells in the body with threatening effects. The incidences of acute myeloid leukemia (AML) in world are about 0.01% and in Pakistan it is more common in Punjab. Commonly, patients with AML were diagnosed by having genetic alterations. Whereas approximately 30% of AML patients have Fms-like tyrosine kinase 3 (FLT3) gene alterations. AML spread very quickly and if not treated it becomes fatal in the short period of time. Through the understanding of molecular biology of cancer and identification of mutations in AML the advancement in the targeted therapeutics has increased and more better drugs, that target products of protein molecules of mutated genes associated with various cancers, are thus being practiced.

Keywords: Acute myeloid leukemia (AML), Fms-like tyrosine kinase3 (FLT3), Internal tandem duplication (ITD).

INTRODUCTION
Leukemia is known when there is an increase number of blast cell due to the abrupt development of immature white blood cells (WBCs) in the bone marrow (Tibes and Noel 2011). The word leukemia is derivation of two Greek words that are "leuko" which means "white" and "haima" which means "blood". A strict equilibrium between self-regeneration and segregation of hematopoietic stem cells is essential in order to sustain homeostasis, as well as to efficiently react to damage and infection because leukemia mainly affects the hematopoietic stem cells (HSCs) and leads to the development of cancer whereas progenitors that develop from HSC and cause incorrect assessment must also be cautiously keeping up to maintain large scale production of blood cells (Luis et al., 2011). In leukemia the irregular appearance of p53, rb and ras genes are interrelated for the production of cytokines which in turn are associated with the abundance of cancerous cells and the irregular proliferation of blood cells (Ashraf and Irshad, 2012). The genetic alterations play a critical function in the leukemia formation and act as oncogenes in tumor disorders (Iqbal, 2012). AML is a diverse ailment with a large number of variations in prediction (Rollig et al., 2011). Acute leukemia accounts for 30% of all childhood malignancies and it is significant to about 15-20% in children having age less than of 14 years (Fadoo et al., 2012).

Types
According to the medical and pathological analyses, leukemia is sub-divided into an array of large assemblage. The initial distinction is involving acute and chronic forms, on the basis that how rapidly it spreads:

Acute leukemia is typified when the amount of undeveloped white blood cells raise suddenly. It spreads very fast and patient shows many clinical symptoms in short period of time (Dores et al., 2010). Whereas, the chronic leukemia is differentiated by unnecessary build up of comparatively mature but still abnormal...
WBCs (Imitola et al., 2012). The disease is also divisible into two types according to which type of blood cells are affected: In lymphoblastic leukemia, the lymphocytes, that are immune system cells and work against diseased cells, acquire cancerous variations (Jansson et al., 2011). Whereas in myelogenous leukemia the bone marrow that forms all type of blood cells become adversely affected due to the leukemia propagation (Reyaund et al., 2011). According to the above classification of leukemia, there are four broad categories of leukemia that are described below:

During the malignancy of AML the alteration occurs in the distinct primordial multipotential hematopoietic cells which change it to a leukemia stem cell. If the ailment is not treated at the proper time than it progresses quickly (Lichtman, 2010). In acute lymphoblastic leukemia (ALL), the development of healthy WBCs lumps in the bone marrow because there is an extreme number of stem cell and lymphoblast (Simone et al., 2010). In different racial groups due to the occurrence of this disease there are many fusion oncogene (Awan et al., 2010). In majority of the developed countries, the five-year survival rates are now greater than 80% for childhood ALL (Yang et al., 2011).

In chronic lymphoblastic leukemia (CLL) there are majority of minute, round, mature-appearing lymphocytes that have unpredictable medical development and appearance (Puente et al., 2011). In adults, it is the most frequently occurring category of leukemia (Fabbri et al., 2011).

In the bone marrow different blood cells propagate abnormally in several myeloid lineages that lead to the formation of chronic myeloid leukemia (CML) (Croce et al., 2010). There are several cytogenetic abnormalities in the CML as one found in the BCR-ABL fusion oncogene that occurs by the mutual translocation of chromosome 9 and 22 and this is known as Philadelphia (Ph) chromosome therefore it is a varied disorder of hematopoietic stem cells (HSCs). Imatinib is frequently used for treatment because the CML patients with BCR-ABL fusion oncogene are efficiently treated with this drug (Gleevec) (Iqbal et al., 2004). The yearly frequency of CML is 1.6/100,000 adults with insignificantly male prevalence (Huang et al., 2012).

Beside these common types of leukemia, there is a distinctive subtype of leukemia known as acute promyelocytic leukemia (APL) in which undeveloped white blood cells called promyelocytes, accumulate in the bone marrow and the uncontrolled growth of promyelocytes cause deficiency of normal blood cells (Kim et al., 2010). The fusion oncogene PML-RARA forms by the fusion of the two genes that are, PML located on chromosome 15 and RARA located on chromosome 17 reveals the translocation t(15;17)(q24;q21) and this fusion is the major reason of APL which found in approximately 92% of the patients (Jie et al., 2012).

**Incidences**

Universally the frequency of leukemia is 0.01% with the annual mortality rate of about 0.008% and it affects adults 10 times more than children (Piller, 2003). The incidence rate of leukemia is higher in males than in females and its also affects more Americans than Afroamericans (Brown et al., 2012). In the industrial countries, cancer is the second important reason of death which accounts for 9.5% of all deaths next to cardiovascular disease which causes 21% of mortality (Hamayun et al., 2005). Mostly the children which are effected with AML have a high chance of early death and median overall survival of nine months (Zaki et al., 2002). AML patients approximately have been reported 19,000 in the United States (Dores et al., 2010). Whereas in Middle East and Iran, it is one of the most frequent type of leukemia (Ahmadi et al., 2012). In national population of Oman, AML with M2-FAB subtype was common that affected about 18% children and 44% adults (Udayakumar et al., 2007).

In Pakistan, annually different kinds of blood cancers are diagnosed in approximately 8000 people, whereas in Karachi the annual report incidences about 800 of the cases (Shamsi, 2012). AML is widespread in the Punjab province than the N.W.F.P and Northern area. Majority (65.4%) of the AML patients in Pakistan fit in average risk groups, while 11.6% belong to poor risk group (Aziz and Qureshy, 2008). The prevalence of childhood leukemia is 5-7 per million people per year and the occurrence remains fixed during infancy with minor increase in puberty (Fadoo et al., 2012). The average age of AML patients ranges from 15 to 70 years and the mean age is 38 years (Kakepoto et al., 2002). In a study reported in Pakistan, it was observed that most of the AML patients were positive for philadelphia chromosome and they were treated with two
generally used drugs that are cytarabine and daunorubicin (Jameel, 2012). Pakistani AML patients have been accounted to have highly developed disease and rates of reduction fluctuating from 65 to 73% (Kakepoto et al., 2002).

**Acute Myloid Leukemia (AML)**

AML is a kind of blood cancer in which the blood cells are not mature normally as these do and the abnormal cells clot in the whole body (Table I) (Levis et al., 2005).

Three projected models used for the predictive stratification of AML, and supported by mutual molecular and cytogenetics criterion (Dohner et al., 2010) or exclusively on molecular constraints (Grossmann et al., 2012) are shown in Table I (Martelli et al., 2013). On karyotype analysis, about 35-50% patients’ lack chromosomal alterations and all of such patients that have cytogenetically typical AML are classified in intermediate risk group (Byrd et al., 2002; Mrozek et al., 2004). In many reported cases of AML, the frequency of males has been reported higher than the females diagnosed with this disease (Ahmadi et al., 2012).

In AML undeveloped WBCs called “promyelocytes” mount up in the bone marrow and fill up the hematopoietic vessels of marrow. Alternatively this variation causes severe damage to the typical blood cell assembly in the marrow which leads to a deep reduction in red cells (anemia), white cells (leucopenia) and platelets or thrombocytes (thrombocytopenia) in the blood (Lichtman, 2010).

**Table I: Revised risk stratification of AML according to different models.**

<table>
<thead>
<tr>
<th>Risk profile</th>
<th>(i) (Dohner et al., 2010)</th>
<th>(ii) (Patel et al., 2011)</th>
<th>(iii) (Grossmann et al., 2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Favorable</td>
<td>t(8,21)(q22;q22); RUNX1-RUNX1T1 inv(16)(p13.1q22) or t(16,16)(p13.1;q22); CBFB-MYH11 NPM1 mutation/FLT3-ITD- CEBPA mutation (*)</td>
<td>Favorable cytogenetics NPM1 mutation/FLT3-ITD- with IDH1 or IDH2 mutations</td>
<td>Very favorable: PML-RARA CEBPA double-mutation</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Intermediate-I: NPM1 mutation/FLT3-ITD- NPM1 wt/FLT3-ITD- (*)</td>
<td>CEBPA mutation FLT3-ITD- and all these genes wild-type: ASXL1, MLL-PTD, PHF6, and TET2 FLT3-ITD+, trisomy 8- negative and all these genes wild-type: MLL-PTD, TET2, and DNMT3A</td>
<td>Favorable: RUNX1- RUNX1T1 CBFB-MYH11 NPM1 mutation/FLT3-TD- CEBPA single-mutation/FLT3-ITD + NPM1 mutation/FLT3-ITD + Wild-type cases</td>
</tr>
<tr>
<td></td>
<td>Intermediate-II: t(9,11)(p22;q23); MLLT3-MLL Cytogenetic abnormalities not classified as favorable or adverse</td>
<td></td>
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</tr>
<tr>
<td>Unfavorable</td>
<td>inv(3)(q21q26.2) or t(3,3)(q21;q26.2); RPN1-EV11 t(6,9)(p23;q34); DEK-NUP214 t(v;11)(v;q23); MLL rearranged - 5 or del(5q); -7; abnormality(17p); complex karyotype</td>
<td>Unfavorable cytogenetics FLT3-ITD+, CEBPA wt and any of these gene alterations: MLL-PTD, TET2, DNMT3A mutations or trisomy 8</td>
<td>Unfavorable: MLL-PTD and/or RUNX1 and/or ASXL1 mutation</td>
</tr>
</tbody>
</table>

(i) Based on cytogenetic analysis and mutation analyses of the NPM1, CEBPA, and FLT3 genes.
(ii) Based on integrated cytogenetic and mutational analysis (MLL-PTD, FLT3-ITD, mutations in NPM1, CEBPA, TET2, ASXL1, DNMT3A, PHF6, IDH1, and IDH2).
Table II: Categorization of acute myelogenous leukemia (WHO 2008).

<table>
<thead>
<tr>
<th>AML with recurrent genetic abnormalities</th>
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<tbody>
<tr>
<td>AML with t(8;21)(q22;q22); RUNX1-RUNX1T1</td>
<td></td>
</tr>
<tr>
<td>AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11</td>
<td></td>
</tr>
<tr>
<td>AML with t(9;11)(p22;q23); MLLT3-MLL</td>
<td></td>
</tr>
<tr>
<td>AML with t(6;9)(p23;q34); DEK-NUP214</td>
<td></td>
</tr>
<tr>
<td>AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1</td>
<td></td>
</tr>
<tr>
<td>AML with mutated NPM1</td>
<td></td>
</tr>
<tr>
<td>AML with mutated CEBPA</td>
<td></td>
</tr>
<tr>
<td>AML with myelodysplasia-related changes</td>
<td></td>
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<tr>
<td>Therapy-related myeloid neoplasms</td>
<td></td>
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<tr>
<td>Myeloid sarcoma</td>
<td></td>
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<tr>
<td>Myeloid proliferations related to Down syndrome</td>
<td></td>
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<tr>
<td>Transient abnormal myelopoiesis</td>
<td></td>
</tr>
<tr>
<td>Myeloid leukemia associated with Down syndrome</td>
<td></td>
</tr>
<tr>
<td>Blastic plasmacytoid dendritic cell neoplasm</td>
<td></td>
</tr>
</tbody>
</table>

Table III: FAB Classification system of AML.

<table>
<thead>
<tr>
<th>FAB subtype</th>
<th>Name</th>
<th>Adult AML patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>Undifferentiated acute myeloblastic leukemia</td>
<td>5</td>
</tr>
<tr>
<td>M1</td>
<td>Acute myeloblastic leukemia with minimal maturation</td>
<td>15</td>
</tr>
<tr>
<td>M2</td>
<td>Acute myeloblastic leukemia with maturation</td>
<td>25</td>
</tr>
<tr>
<td>M3</td>
<td>Acute promyelocytic leukemia</td>
<td>10</td>
</tr>
<tr>
<td>M4</td>
<td>Acute myelomonocytic leukemia</td>
<td>20</td>
</tr>
<tr>
<td>M4eos</td>
<td>Acute myelomonocytic leukemia with eosinophilia</td>
<td>5</td>
</tr>
<tr>
<td>M5</td>
<td>Acute monocytic leukemia</td>
<td>10</td>
</tr>
<tr>
<td>M6</td>
<td>Acute erythroid leukemia</td>
<td>5</td>
</tr>
<tr>
<td>M7</td>
<td>Acute megakaryocytic leukemia</td>
<td>5</td>
</tr>
</tbody>
</table>

AML also frequently found in children and it is related with some inherited disorders, such as if children have Down syndrome than the probability of developing acute leukemia enhances about 10-20 folds, other disorders linked with AML are Klinfelter’s syndrome, fanconi anemia and neurofibromatosis (Asif et al., 2011). Overall about 80% of all the adult leukemic patients are suffered from AML (Pollyea et al., 2011) (Table II, III).

CLASSIFICATION OF AML
There are two most commonly used classification systems for AML:

**World Health Organization (WHO) System**
This classification system uses all accessible information, morphology, cytochemistry, immunophenotype, heredity and medical features to classify clinically important disease components (Cazzola et al., 2011). The World Health Organization (WHO) in conjunction with the society for Haematopathology and European Association of Haematopathology published a new classification for hematopoietic and lymphoid neoplasm and set up grouping for AML with discrete cytogenetic and more consistent medical activities. Table II reviews the new categorization of AML as proposed by World Health Organization (WHO), (Vardiman et al., 2009).

**French – American – British (FAB) System**
The FAB classification primarily proposed in 1976 that presents an
understanding for the reliable morphologic and cytochemical organization which can be used for the implication of the genetic laceration. The FAB classification system divides AML into 8 sub-types, from M0 to M7 (Seiter and Harris, 2011). FAB classification system of AML and its cell-surface and cytoplasmic indicators have been depicted in tables III and VI.

**Signs, symptoms and causes of aml**
The medical signs and symptom are fever due to the attack of some viruses, the malfunctioning of immune system to react because of the reduction in the amount of healthy WBCs, bleeding and anemia due to the damage of platelets and weakness, abdominal pain, nausea, annoyance, sickness and manifestation of the small spots on the skin (Aziz and Qureshy, 2008). It has become evident that difference in the appearance of genes contributes to pathogenesis (Udayakumar et al., 2007). Immunophenotyping using multiparameter flow cytometry to establish extraction involvement of a newly diagnosed acute leukemia (Bene et al., 1995; Craig and Koon, 2008; Sanz et al., 2009). The two important gene alterations that occur in AML patients are nucleophosmin-1 (NPM-1) and Fms-like tyrosine kinase 3 (FLT3) genes (Ghosh et al., 2012). Due to benzene exposure, mutations occur in a critical gene or a set of genes related to propagation and segregation in hematopoietic stem cells (HSCs) in the forms of chromosomal abnormality, deviant mitotic recombination and epigenetic alterations and have been associated with AML disease (Smith et al., 2004). Infants that have Down syndrome also have a unique partiality to develop acute myeloid leukemia (Gamis et al., 2011). Among adults a few viruses such as “Human T-Lymphotropic” virus, natural and artificial “Ionizing radiations” and also smoking were identified as the causes for earlier disorder (Estey, 2012). The defects in a group of proteins are responsible for DNA repair mechanism cause Fanconi’s anemia that is a genetic disease and can also lead to the development of AML (Mushtaq et al., 2012). If a pregnant woman uses teratogens during the 2 month after conception, which is the stage of organ formation than this would also lead to the growth of AML (Cardonick and Iacobucci, 2004).

**Table IV:** Appearance of cell-surface and cytoplasmic indicators for the analysis of acute myeloid leukemia (Dohner et al., 2010).

<table>
<thead>
<tr>
<th>Appearance of indicators for diagnoses</th>
<th>Prediction of acute myeloid leukemia (AML)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prerucrursor stage HLA-DR</td>
<td>CD34, CD38, CD117, CD133</td>
</tr>
<tr>
<td>Granulocytic indicators cytoplasmic myeloperoxidase (cMPO)</td>
<td>CD13, CD15, CD16, CD33, CD65</td>
</tr>
<tr>
<td>Monocytic indicators</td>
<td>Nonspecific esterase (NSE), CD11c, CD14, CD64, Isozyme, CD4, CD11b, CD36, NG2 homologue‡.</td>
</tr>
<tr>
<td>Megakaryocytic markers</td>
<td>CD41 (Glycoprotein Iib/Iiia), CD61 (Glycoprotein Illa), CD42 (Glycoprotein Ib).</td>
</tr>
<tr>
<td>Erythroid indicator</td>
<td>CD235a (Glycoprotein A)</td>
</tr>
</tbody>
</table>

*For the analysis of AML, the table presents a record of ideal markers instead of a mandatory marker division.
‡Most cases with 11q23 abnormalities exhibit the presence of NG2 homologue (encoded by CSPG4) which reacts with the monoclonal antibody 7.1.

**Fusion oncogene**
Around 200 fusion oncogenes have been described in human tumors. Fusion oncogenes form a functional transcriptional element that consists of full length or part of two genes. Oncogenes are normally found in healthy individuals and any type of mutation can lead to the formation of tumor and propagation of cancer, mixed lineage leukocyte (MLL). Whereas fusion oncogenes arise more commonly from cryptic genetic rearrangements and are often caused by mutation or over expression of FLT3 (Ono et al., 2009). AML/MTG8 is one of the most commonly found leukemic fusion oncogene which is associated with AML (Gessner et al., 2010). A specific transcription that yields a 90 to 110 KD protein, so that the product of third exon which was present on chromosome 17 of the RAR gene will fuse with the amino terminal position of Zn-finger protein and PML from chromosome 15, forms PML-RARα-A and PML-RARα-B by alternative breakpoints in cells of about 90% APL patients (Martens, 2011).
**Mutation**

By the occurrence or lack of alterations in further genes, the incidence of one gene mutation can be stratified (Schlenk et al., 2008). Although for the finding of AML the clinical symptoms and prognostic factors are useful but the analysis of cytogenetic abnormalities are valuable for the diagnosis and treatment of AML due to two reasons that is to verify the exact type of AML and to choose the accurate treatment for each patient (Aziz and Qureshy, 2008). Two foremost groups of genetic abnormalities have been found in AML patients: Group 1 include genetic alterations that result in an endurance benefit and propagation of blood stem cells and group 2 includes genetic aberrations that cause blood forming cells to lose their capability to distinguish and go through apoptosis; the association was required among Group 1 and Group 2 for the development of AML (Kelly and Gilliland, 2002). Based on the genetic and microRNA expressions, many new sub-classes are also budding (Bacher et al., 2009; Larson, 2010).

**Table V: Genetic transformations that concern prediction in patients with Acute Myeloid Leukemia.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Definition</th>
<th>Expression</th>
<th>Prognostic Effect</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM-1</td>
<td>Nucleophosmin-1</td>
<td>Mutation</td>
<td>Favorable: Higher CR rates; better OS, EFS, and DFS</td>
<td>Dohner, 2005; Schnittger, 2005; Thiede, 2006.</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>Fms-like tyrosine kinase receptor internal tandem deletion</td>
<td>Mutation</td>
<td>Unfavorable: Worse DFS and OS</td>
<td>Whitman, 2001; Thiede, 2002; Frohling, 2002; Ciolli, 2004; Stirewalt et al., 2006.</td>
</tr>
<tr>
<td>FLT3-PM</td>
<td>Fms-like tyrosine kinase receptor point mutation</td>
<td>Mutation</td>
<td>Unclear</td>
<td>Frohling, 2002; Kiyoi, 2006; Mead, 2007; Whitman, 2008.</td>
</tr>
<tr>
<td>FLT3</td>
<td>Fms-like tyrosine kinase receptor</td>
<td>Overexpression</td>
<td>Unfavorable: Worse OS and greater resistant disease</td>
<td>Ozeki et al., 2004; Kang et al., 2010.</td>
</tr>
<tr>
<td>BAALC</td>
<td>Brain and acute leukemia, cytoplasmic</td>
<td>Overexpression</td>
<td>Unfavorable: Worse DFS and OS; greater resistant disease</td>
<td>Baldus et al., 2006.</td>
</tr>
<tr>
<td>MN1</td>
<td>Meningioma 1, disrupted in balanced translocation</td>
<td>Overexpression</td>
<td>Unfavorable: Poor response to treatment, high relapse rate, worse risk-free survival and OS</td>
<td>Heuser et al., 2006.</td>
</tr>
<tr>
<td>MLL-PTD</td>
<td>Mixed-lineage leukemia partial tandem duplication</td>
<td>Mutation/overexpression</td>
<td>Unfavorable: Lower remission durations, worse median survival and relapse-free intervals</td>
<td>Schnittger, 2000; Dohner, 2002; Weisser et al., 2005.</td>
</tr>
<tr>
<td>CEBPα</td>
<td>CCAAT/enhancer-binding protein alpha</td>
<td>Mutation</td>
<td>Favorable: Better EFS, DFS, and OS</td>
<td>Frohling et al., 2004; Bienz et al., 2005.</td>
</tr>
<tr>
<td>ERG-1</td>
<td>ETS-related gene-1</td>
<td>Overexpression</td>
<td>Unfavorable: Worse OS, greater relapse</td>
<td>Marcucci et al., 2005.</td>
</tr>
<tr>
<td>IDH-1</td>
<td>Isocitrate dehydrogenase-1</td>
<td>Mutation</td>
<td>Unfavorable: Worse DFS, higher risk of relapse</td>
<td>Marcucci et al., 2010; Boissel et al., 2010.</td>
</tr>
<tr>
<td>IDH-2</td>
<td>Isocitrate dehydrogenase-2</td>
<td>Mutation</td>
<td>Unfavorable: Lower remission rates, shorter OS, higher risk of induction failure</td>
<td>Marcucci et al., 2010; Boissel et al., 2010.</td>
</tr>
<tr>
<td>WT-1</td>
<td>Wilms’ tumor-1</td>
<td>Mutation</td>
<td>Unfavorable: Shorter OS, lower CR, higher relapse rates, shorter DFS</td>
<td>Paschka et al., 2008; Renneville et al., 2009; Becker et al., 2010.</td>
</tr>
</tbody>
</table>

CR indicates complete response; OS, overall survival; EFS, event-free survival; DFS, disease-free survival.
The patients that have genetic abnormalities of intermediate risk group, led to changes in the appearance of gene such as aberration and polymorphism which are important for the prediction, to assist thorough treatment and these definite alterations also appropriate for therapeutic targets (Table V) (Pemmaraju et al., 2011).

Tyrosine kinase, Kit and FLT3 are two basic classes of growth factor receptors which cooperate an important role in propagation of AML ( Muller et al., 2004). On Asp 816 Tyr activating mutation in an AML-M2 with (8; 21) occurring and later this link strengthening by Asp 816 mutations in 4/9 patients with (8; 21) and 2/6 patients with inversion (16) has been documented (Beghini et al., 2000). In AML patients the most part of recurrent somatic alterations are of FLT3 gene mutations, occurring in about 1/3 of patients followed by RAS and TP53 genes that play important roles in the regulatory processes that direct propagation, segregation and apoptosis (Kelly and Gilliland, 2002; Mukherjee et al., 2000) and the pathogenesis of younger adult patients of AML have been concerned with the abnormalities of these three genes. Just about 20% of de novo AML patients have point mutation that occurs in RAS oncogenes (Mckenna et al., 2000). Genetic abnormalities that include translocation, inversion, amplification, deletion, and insertion are identified to contribute leukemia (Mitelman et al., 2004). As the disturbance in the segregation, division and the regulation of cell death in the FLT3 gene generate AML, similarly certain mutations by activating “oncogenes” or deactivating “Tumor suppressor genes” cause AML. In cancer expansion there are about 293 genes that have been concerned (Falini et al., 2005). A tyrosine kinase “Janus kinase 2” (JAK2) was implicated in the stimulus transduction of cellular maturity which due to the chromosomal translocation causes in fusion deregulating JAK2 activity which is concerned with the development of leukemia (Schnittger et al., 2005). The AML which have inferior to myeloproliferative disorders, myelodysplasia and therapy-related AML, infrequently show cytoplasmic NPM1 mutation, therefore NPM1 mutation intimately is related with de novo AML (Falini et al., 2005). The detection of NPM1 mutations in AML is particularly associated with chromosomal abnormalities that are principally pathogenic changes, the simple observation of bone marrow biopsies at microscope from about one-third of AML patients shows the ectopic expression of nucleophosmin in the cytoplasm of cancerous cells which led to the recognition of NPM1 mutations in AML (Mrozek et al., 2004). Depending on gene dosage, expression levels, interacting partners and compartmentalization, NPM1 function both as oncogene and tumor suppressor genes (Nafea et al., 2011).

Myeloid neoplasms are distinguished by attaining somatic and epigenetic alterations in genes that are vital for blood cells’ segregation, propagation and survival (Odenike et al., 2011). Subsequent to NPM1 is C-Kit gene that is the most generally mutated gene in AML patients (Dombret, 2011). In 15.68% (8/51) AML patients, the c-Kit mutation was detected on exon 11 (Hussain et al., 2011). Whereas about 10-15% patients of AML have certain translocations and inversions, such as t(8;21)/runt-related transcription factor 1 (RUNX1)RUNX1 translocated to 1 cyclin D-related (RUNX1-RUNX1T1), the abnormal core-binding factor (CBF) mutations, t(15;17) promyelocytic leukemia-retinoic acid receptor q (PML-RARA) and inversion 16/core-binding factor β-myosin heavy chain 11 smooth muscle (CBFB-MYH11) (Pemmaraju et al., 2011). The AML mutations are distinct according to their medical and predictive impacts (Martelli et al., 2013; Hatzimichael et al., 2013).

Mutations with widely recognized clinical impact

a- FLT3 gene mutation

FLT3 has a significant function in scheming usual blood cells formation, cells growth in primordial hematopoietic and progenitor cells (Adolfson et al., 2005). In human this protein is encoded by the FLT3 gene that is located on chromosome 13q1 (Bains et al., 2011). FLT 3 contains 24 exons and 993 amino acids which are expressed on the surface of hematopoietic progenitor cells (HPCs) as a “cytokine receptor”, which is also known as “Fetal liver kinase 2”, “FMS-like tyrosine kinase3” and “cluster of differentiation 135 (CD135). CD135 is a central cell surface indicator used to categorize certain types of hematopoietic progenitors in the bone marrow. It is expressed in immature hematopoietic cells, placenta, gonads, brain and in lymphohematopoietic organs such as the spleen, thymus and the liver (Hatzimichael et al., 2013). When FLT3 gene is stimulated by mutation then
it results in uncontrolled phosphorylation as well as normal phosphorylation of many enzymes either directly or indirectly. As a result of constitutive activation of FLT3 variations of gene appearance have been revealed by microarray or other expression leukemic cell lines (Berdel et al., 2005). When FLT3 ligand (FL) bind to the monomeric unphosphorylated receptor of FLT3 then it starts a series of reactions which causes unfolding, homodimerization and autophosphorylation of FLT3, resultanty the tyrosine kinase activity switches on and many intracellular proteins recruits to its intracellular domain, these proteins are activated and then start a cascade of phosphorylation which then activates secondary mediates such as phosphoinositide 3-kinase (PI3 K), AKT, mitogen activated protein kinase (MAPK) and signal transducer, activator of transcription (STAT), and MAP kinase that are transported to the nucleus through HSP90; where they start transcription of various genes that helps in proliferation, differentiation and apoptosis (Fig. 1) (Hatzimichael et al., 2013). One of the genetic alterations that are frequently observed in AML patients are in the FLT3 gene (Pemmaraju et al., 2011). The finding of FLT3 mutation is an influential discovery that confirms the significance of FLT3 gene in leukemia (Shen et al., 2011). The patients with AML have highly (80%) expressed FLT3 protein (Ishfaq et al., 2012). Due to the FLT3 gene alteration higher bone marrow blast and intricate cytogenetic features have been related with development of AML (Asif et al., 2011). About 25% to 30% patients with AML have transformation of FLT3 gene (Kotzaridis et al., 2001; Frohling et al., 2002). It has also been considered that in AML, FLT3 mutations are derivatives and not initiate the genetic abrasion (McKormic et al., 2010). Actively expression of FLT3 on leukemic cells of about 40% patients that were clinically diagnosed to have AML, has been documented in which the mutated gene caused proliferation and survival of leukemic blasts (Kelly et al., 2002). A division of effected cells surrounded by a cancer is responsible for the diffusion of disease. In case of leukemia these cells have been termed as "leukemia stem cells (LSCs)". LSCs frequently allocate lots of the uniqueness with HSCs but are not essentially derived from them; however, at least, FLT3 gene mutation occurs in LSCs. For the propagation of leukemia FLT3 gene mutation cooperates with other gene transformations such as NPM1 as well as DNMT3A and in this way these genetic determinants play a complex role in leukemogenesis (Ley et al., 2010; Falini et al., 2011). FLT3 gene mutation principally affects two major regions of enzyme that are juxtamembrane (JM) part and activation loop of tyrosine kinase domain (TKD) (Dohner and Gaidzik, 2011). Usually FLT3 gene has two types of mutations that are internal tandem duplication (ITD) and point mutation. Alteration in the juxtamembrane region is known as ITD mutation and the abnormality in activation loop is called as KD point mutation. Both types of those mutations result in lack of tyrosine kinase receptor function in the ligand (Griffith et al., 2004). Point mutations occur in tyrosine kinase and juxtamembrane domain (Ghosh et al., 2012). Due to point mutations, the minute insertions and deletions which generally involve codons 835 and 836 affect the tyrosine kinase domain of FLT3 gene (Dohner and Gaidzik 2011). The poor predictive assessment of FLT3/ITD mutation was linked with high early ailment decline and generally shorter survival (Govedarovic and Marjanovic, 2001). Internal tandem duplication in AML patients was first recognized by Nakao and his followers in 1996 (Martelli et al., 2013). About 25% of patients with AML have alteration of internal tandem duplication (ITD) (Mead et al., 2007) (Fig. 1). Due to the above referred duplications, 3 to greater than 100 extra amino acids might be added in the receptors of FLT3 gene (Schnittger et al., 2002). Until now ITDs have been reported merely in the juxtamembrane domain of the enzyme but much of the lab efforts had showed that 34% of ITD alterations also arise inside the tyrosine kinase domain (Breitenbuecher et al., 2009) (Fig. 2). Within AML patient’s frequent cytogenetic abnormalities were found but it is not necessary that FLT3-ITD mutations are equally restricted to AML (Falini et al., 2008). FLT3-ITD alterations were typified to have great difference as compared to mutational weightage that was expressed as FLT3-mutant/wild-type allelic proportion, localization and size (Gale et al., 2008; Schnittger et al., 2012). The greater part of many research woks have revealed that people suffering from AML have high death rate with a high fraction FLT3-mutant to wild-type than those with inferior fraction (Gale et al., 2008; Schnittger et al., 2011; How et al., 2012). The resultant destructive outcome of FLT3-ITD mutation is that it stimulates abandoned production of the leukemia blast cells (Fathi et al., 2010) and is also related with elevated rate of WBCs (Kutny et al., 2012).
ROLE OF FLT3 GENE IN MYELOID LEUKEMIA PATIENTS

Figure 1: Basic diagram of stimulus downstream flow of FLT3 that are considered supportive to leukemia formation (Fathi and Chabner, 2011).

Abbreviations: BAD, Bcl-2-associated death promoter; ERK, extracellular signal–related kinase; FL, FLT3 ligand; FLT3, FMS-like tyrosine kinase 3; Grb2, growth factor receptor-bound protein 2; MEK, mitogen-activated protein kinase/ERK kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PIM1, proto-oncogene serine/threonine-protein kinase 1; PIP2, phosphatidylinositol-bisphosphate; PIP3, phosphatidylinositol-trisphosphate; Rheb, Ras homolog enriched in brain; SOS, son of sevenless; STAT-5, signal transducer and activator of transcription 5; TSC, tuberous sclerosis protein.

Figure 2: Diagrammatic representation of flt3 gene itd and kd domain (Small, 2006).
As a result of such detrimental effects, these mutations have a high risk of decline and death (Estey, 2012). The most frequent transformation of FLT3 gene is internal tandem duplication (ITD) which arises at 3-400 base pairs that figures to juxtamembrane region. About 31% of patients with AML have ITD mutation of FLT3 receptor tyrosine kinases that result in constitutive kinase activation by the duplication of specific portion within juxtamembrane coding region and are considered to be diagnostically associated properly FLT3, like a reasonable curative intention (Gianfelici et al., 2011). However, FLT3/ITD mutation is still present in AML patients when relapse takes place (Shih and Huang, 2002). The less frequent transformation of FLT3 gene is point mutation. Mainly point mutation occupy a portion of tyrosine kinase domain TKD that comprises “Aspartic acid” 835, but sometimes it can also be found at several other sites (Chauhan et al., 2011). In 16% of AML cases, when relapse occurs the FLT3 gene mutation was no longer present, but in more than 50% of AML patients the situation was diverse for FLT3-TKD mutations which were lost at relapse (Shih et al., 2004). FLT3/TKD causes receptor activation and autophosphorylation through propagation which activates downstream effectors and generates diverse genetic reactions (Grunder et al., 2005; Choudhary et al., 2005). In numerous research works it has been confirmed that FLT3-ITD has harmful impact on diagnosis (Whitman et al., 2001; Thiede et al., 2002). The negative predictive effect of FLT3-ITD has been observed in many patients with different age groups and these patients varied in age from newborn to mature adults of greater than 58 years and all had harmful prediction compared to other patients that do not had FLT3-ITD mutations (Meshinchi et al., 2006; Breccia et al., 2009). In mutated AML patients, the high rate of survival and complete hematological response can be achieved by the appropriate treatment of FLT3-ITD alteration (Man et al., 2012).

Number of patients of FLT3 gene mutation

A variety of report studies about AML mutations show important variations which were significant to check the survival in patients of all ages due to the existence of ITD alteration. For example, in one study it was checked that the survival rate with no event of death, was 45% for patients without a FLT3/ITD mutation as contrasted to those that had FLT3/ITD alteration with survival rated only about 7% (Meshinchi et al., 2001). On the whole the prevalence of ITD alteration was subordinate just about 15% in children with AML, while FLT3 mutation occurred more recurrently in about 5-22% ALL and Hyper diploid patients (Armstrong et al., 2003, 2004). FLT3/ITD was infrequent in infant AML patients and increase up to 5% to 10% in patients of age groups 5-10 years, in young adults 20% and patients older than 55 years had its chance of >35% (Stirewalt et al., 2006).

b- NPM1 Mutation

NPM1 (nucleolar phosphoprotein, nucleophosmin 1) makes nucleophosmin protein, which is also identified as B23 or N038 and is extensively expressed in a variety of cells that transport among nucleus and cytoplasm (Chen and Lu, 2013). It renovates the protein localization, aggregation and constancy of the tumor suppressor p53 and p14ARF (Liu et al., 2012). This protein also plays a part in cellular functions, together with the production of ribosomes and their export, centrosome repetition, DNA repair, chromatin modification and also responses to pressure stimuli (Federici and Falini, 2013). NPM1 mutation is considered as the initiator of genetic alterations in AML in the course of diverse verifications (Falini et al., 2011). It is the most recurrently acquired molecular genetic abnormality in 30% AML patients particularly that have regular karyotype (Schnittger et al., 2009). Insertion mutations in exon 12 at C-terminus of the NPM1 gene cause unusual cytoplasmic existence of the nucleophosmin protein and have been recognized in about half of AML cases (Sockel et al., 2011; Balusu et al., 2011). In many of the adult AML patients it is the most frequent single gene irregularity and accounts for 50-60% in usual cytogenetic AML (Marcucci et al., 2011). In AML patients with cytogenetic normal karyotype the incidence of this alteration reduces with age (Schneider et al., 2012). It has been reported that in AML patients that are mutated with NPM1 gene, the demethylating agent 5-azacitidine (5-aza) can be used as a drug (Wermke et al., 2010; Sockel et al., 2011).

c- CEBPA Mutation

CCAAT/enhancer binding protein alpha gene (CEBPA) present on band q13.1 of chromosome 19 encodes the essential area of leucine zipper (bZIP) transcription factor which is
concerned in myelopoises, proliferation, neutrophil maturity and organized terminal granulocyte differentiation (Lekstom-Himes and Xanthopoulos 1998; Schlenk et al., 2008). It is predominantly promoted throughout granulocytic differentiation and is present in myelomonocytic cells (Leroy et al., 2005). In AML, CEBPA is divided into two main types: the first type found on N-terminal which causes frame shift mutations and eliminate the complete extended transformation of CEBPA protein (p42 CEBPA), leading to the over appearance of the principal unconstructive 30-kDa that is almost transformed structure of CEBPA (p30 CEBPA) (Wouters et al., 2009). The second kind of CEBPA mutation is present on C-terminal and this alteration occurs within coding region of the bZIP domain of CEBPA and leads toward the production of such protein that has decreased homo-dimerization and hetero-dimerization and damages DNA obligatory actions (Asou et al., 2003). It has been accounted that 10-15% of clinically diagnosed AML patients have CEBPA gene alterations and about one-third of such AML patients have a single CEBPA mutation (CEBPAsm). Whereas the remaining two-third AML patients have a single CEBPA mutation and about one-third of such clinically diagnosed AML patients have CEBPA gene alterations and about one-third of such AML patients have a single CEBPA mutation (CEBPAdm). On one allele of these patients N-terminal mutation is present whereas the second allele has C-terminal mutation (Dohner and Gaidzik 2011).

Mutations in epigenetic modifiers genes: a- IDH1 and IDH2 Mutations

These are the NADP-dependent isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2) genes and are present in cytosol where it they catalyze a reaction in the citric acid cycle (Reitman and Yan, 2010). There are mainly three subsisted categories of IDH iso-enzymes that is mitochondrial NAD-dependent IDH (mt-NAD-IDH), mitochondrial NADP-dependent IDH (mt-NADP-IDH) and cytosolic NADP-dependent IDH (cy-NADP-IDH). Human mt-NAD-IDH1 enzyme is formed by IDH1 gene present at chromosome band 2q33.3 which is present in cytoplasm and peroxisomes and the mt-NADP-IDH2 enzyme is encoded by the IDH2 gene, present on chromosomal band15q26.1 (Paschka et al., 2010). In AML, IDH1 and IDH2 mutations are usually restricted to some definite points of gene (Rakheja et al., 2012). IDH1 mutation was initially discovered from a CN-AML patient (Mardis et al., 2009). In human, the codon R132 forms arginine factor that attaches with the isocitrate and this one is the basic point that is affected by the IDH1 alterations during the disease (Paschka et al., 2010).

This mutation has low rate of occurrence and represents about 10-13% of CN-AML cases. (Schnittger et al., 2010; Abbas et al., 2010; Patel et al., 2011). The regularity of IDH1 mutation is usually low in pediatric AML patients (Damm et al., 2011). It is also examined that in some patients that have CN-AML, IDH1R132 mutation is coupled with the NPM1 mutations and its outcome is the progression of AML (Abbas et al., 2010; Paschka et al., 2010; Green et al., 2010). IDH1 mutation was reciprocally selected with TET2 mutations (Figueroa et al., 2010). IDH2 was originated sometime in about 9-11% of cytogenetically normal AML patients and affects codon R172 (Marcucci et al., 2010; Abbas et al., 2010; Dohner and Gaidzik 2011). IDH2 alteration was also reciprocally selected with other mutations and also bunch with NPM1 mutations (Rakheja et al., 2012).

b- DNMT3A Mutation

DNA methylation in human is completed by three categories of DNA methyltransferase genes that are DNMT1, DNMT3A and DNMT3B. These genes encode some enzymes so as to transmit methyl group onto the 5’ position of cytosine at CpG dinucleotides (Bestor, 2000). DNMT3A transformation causes down-regulation of methylation (Jurkowska et al., 2011). It is also basis of up-regulation of HOXA7, HOXA9 and HOXA10 genes in AML patients that have NPM1 transformation (Yan et al., 2011). Mostly its rate of incidence in AML patients is about 20% (Hou et al., 2012). However, according to the implication of the racial environment in Asia, the rate of recurrence of this gene alteration has been documented as lower as 0.10% (Li et al., 2012). DNMT3A mutation is restricted with alterations that affect the genes NPM1, IDH1 and FLT3-ITD (Thol et al., 2011; Ribeiro et al., 2012).

c- TET2 Mutation

Alteration in ten-eleven-translocation-2 or tet oncogene family member 2 (TET2) was first revealed in myeloid lineage disorders (Chou et al., 2011). TET2 protein also has a role of enzyme that catalyzes translation of methylcytosine to hydroxymethylcytosine, with ferrous iron and α-ketoglutarate as cofactors (Ko et al., 2010). It has been reported that about 7.6% of AML patients have TET2 mutations (Gaidzik et al., 2012).
d- **MLL mutation**
Mixed lineage leukemia gene (MLL) is histone methyltransferase and transcriptional co-activator that plays an important function in early progression and hematopoiesis (Scharf et al., 2007). Typically the propagation of leukemia is dependant on peculiar appearance of MLL gene (Krivstov and Armstrong, 2007). In AML cases the initial alteration was observed in MLL gene with partial tandem duplication (PTD) which consists of in-frame duplication of MLL exons that was named due to the combined exons of e9/e3 (Hatzimichael et al., 2013). The prevalence of MLL-PTD has been reported 6-8% in AML whereas in case of trisomy-11 occurrence rate, has been documented upto 25% (Marcucci et al., 2011).

Less common mutations:
a- **WT1 mutation**
Due to the reduced prediction and lesser outcome the scientific implication of this gene alteration has been linked with medium survival (Paschka et al., 2008). This transformation has been demonstrated in almost 10-13% of AML patients (Gaidzik et al., 2009).

b- **RUNX1 mutation**
This alteration is present on terminal point of the gene with trisomy of 13 and 21 (Schnittger et al., 2011). The rate of recurrence of this gene in AML cases vary from 6-24% (Gaidzik et al., 2009).

c- **BCOR mutation**
Initially by the entire genome sequencing of an AML patient BCOR mutation has been revealed (Grossmann et al., 2011). This transformation proceeds by impeding with the epigenetic methods (Tiacci et al., 2012). It comes in 4% of AML patients (Grossmann et al., 2011).

d- **BAALC Mutation**
In AML, the brain and acute leukemia cytoplasmic (BAALC) gene alteration comes out to be imperative in many of the AML patients (Eisfeld et al., 2012). This gene is basically found on chromosome 8q22.3 (Baldus et al., 2006). The AML patients that have low and high expression of BAALC gene mutation, have comparable or elevated level of WBCs and normally belong to the M4 and M5 FAB category of AML (Bienz et al., 2005). FLT3 mutation basically in the case of FLT3-ITD mutation will compensate the predictive expression of BAALC level of appearance (Motyckova and Stone, 2010).

e- **ERG Mutation**
ETS-related gene (ERG) located on chromosome arm (21q22) and is imperative for cell propagation, segregation and apoptosis (Marcucci et al., 2005). In AML, it is concerned with cytogenetic, molecular reshuffling and its elevated appearance is related with reduce CR (Marcucci et al., 2007).

**TREATMENT**
The AML patients treated with the combined drug therapy that is cytarabine and daunorubicin with the selected dose of 45mg/m2 were first reported in 1973, and the treatment strategy was called as “7 and 3 DNR 45” (Yates et al., 1973). In 1981, the “Cancer and Leukemia group B (CALGB)” verified the dominance of daunorubicin with the dose of 45mg/m2 as compared to the combination of daunorubicin and adriamycin, however, in 1990s daunorubicin was replaced with the combination drug therapy of idarubicin and mitoxantrone with selected doses of 12mg/m2 for both (Roboz, 2011). The patients falling in <60 years of age which were mostly treated through chemotherapeutic drugs showed about 60-80% reduction rate of their disease but following the resistance in the patient’s body against these drugs the survival rate declined to about 30% and became poorer when such a resistance occurred in elder patients with age greater than 60 years, because such patients cannot tolerate intense treatment (Brown and Hughes, 2012). Even though the patients of AML that are treated with the Ara-C and Anthracyclin drugs with the addition of other improved and advanced supportive care show better response outcome but instead of this improvement the frequency of relapse also remains high and is the main cause of death (Ameri et al., 2010).

In spite of many modern advances in the field of treatment, the cure of patients with AML stay behind challenging and complicated situations and there is a major objective to come up at the improvement of novel drug treatment of AML that can enhance the antileukemic effects (Altman et al., 2011). AML patients are conventionally treated so that first they can achieve remission then are managed towards attaining induction and post-remission stage (Kantarejain et al., 2006). For remission and consolidation mostly the combination of ara-C and anthracyclin drugs are used (Roboz, 2011).
A specific category of antibodies, known as Blinatumomab is a definite single-chain antibody that targets the CD19 antigen, is used as a treatment strategy that promotes t-cells for the careful lysis of cancerous cells (Kufer et al., 2011). When chemotherapeutic treatment not proved effective then hematopoietic cell transplantation (HCT) can also be used for the treatment option of AML patients but it is not an alternative for many elderly patients (Armand et al., 2012). In Pakistan, the most commonly used drugs are daunorubicin and cytarabine which have better effect on survival rate (Aziz and Qureshy, 2008).

After the drug treatment of AML patients, if they uphold <5% blasts in their bone marrow, >1000 neutrophil and >100,000 platelet then it is considered that patients are attaining cure and survival. Undoubtedly the essential principle of drug treatment is to uphold complete remission (CR) (Ashraf and Irshad, 2012). Most of the survival trials and response criteria are extensively used by medical experts and accommodating groups (Table VI). Mostly the AML patients are treated with an anthracyclin, such as daunorubicin (cerubidine) or idarubicin (idamysin) and cytarabine (cytosar U) given for three and seven days, respectively (Ashraf and Irshad, 2012). In addition with the above mentioned drugs, sometimes melphalan is used for treatment which is extremely efficient and tolerated by patients (Whittle et al., 2013). Some of the superlatively typified therapeutics that can also be used for AML treatment includes CEP-701 (Lestauntinib), PKC 412, MLN518 and Sunitinib (Levis et al., 2002; Farrell et al., 2003). MLN518 (formally known as CT53518) is a small molecule that can slow down the autophosphorylation activity of FLT3, KIT and platelet derived growth factor receptor (PDGFR) tyrosine kinase with important action in murine form of FLT3/ITD affirmative leukemia (Volkots et al., 2002). Although the collaborations between intellectual and many medical experts have explored a great number of FLT3 inhibitors, but the progress in new drugs to restrain the FLT3 kinase domain was the Tyrosine Kinase Inhibitors (TKI) (Levis et al., 2005). Anti-FLT3 Antibody is a further important approach to target exclusively FLT3 gene. These antibodies can be generated from fully human phage that display such antibodies which can bind to human FLT3 gene with the capability to obstruct FLT3 Ligand (FL) that binds to FLT3 gene to stimulate it (Piloto et al., 2006). At the present time, single nucleotide polymorphism arrays (SNP-A) are in exercise as an influential genotyping tool for an assortment of whole-genome involvement studies (Maciejewsky et al., 2008). Beside many important drugs, sorafenib has been accounted to illustrate important activities against FLT3 (Zhang et al., 2004, 2008; Metzelder et al., 2009).

In cases with low blast percentages (5-10%), a repeat marrow should be performed to confirm relapse. Appearance of new dysplastic changes should be closely monitored for emerging relapse. In a patient who has been recently treated, dysplasia or a transient increase in blasts may reflect a chemotherapy effect and recovery of hematopoiesis. Cytogenetics should be tested to distinguish true relapse from therapy-related MDS/AML.

Belinostat that is a histone deacetylase inhibitor in combination with anthracyclin when used as in vitro has confirmed efficient cell assassination in leukemic cells and illustrates a synergistic outcome (Bullinger et al., 2012). An anti-FLT3 monoclonal antibody known as IMC-EB10 is also under development because by binding to the receptor it can block signaling inducing antibody-dependant cell mediated cytotoxicity and the effects of IMC-EB10 against propagation activity of FLT3-AML replica have been established through the preclinical studies (Youssoufian et al., 2010). FLT3 inhibitors when used singly and with supplementary drugs show greater activity in relapse refractive patients and also in newly diagnosed patients (Pemmaraju et al., 2009). The gene identified as multidrug-resistance-associated protein (MRP) is concerned in the mechanism of resistance to chemotherapy that is also the carrier of glutathione and lung resistance protein (LRP) composite (Ashraf and Irshad, 2012). Chemotherapy for AML also affects usual cells of the body and causes bruising, exhaustion, bleeding, nausea and lowers body’s ability to fight against disease, also causes hair loss and affects patient’s fertility too (Janthur et al., 2012).

**DRUG TREATMENT**

It is considered that in AML cases, the FLT3 tyrosine kinase is the most realistic targeted protein (Fathi and Chabner, 2011). Originally small molecular inhibitors were used to target FLT3 mutations in AML, which were basically demonstrated to be ineffective and resulting only in temporary reducion in peripheral blasts with little response of bone marrow (McCormik et al., 2010). Many FLT3 tyrosine kinase inhibitors...
have been reported for the treatment of AML patients but occasionally derivative mutations crop up within the mutated gene itself and cause resistance against the FLT3 tyrosine kinase inhibitors throughout the course of treatment which in turn limits the prospective assistance of FLT3 tyrosine kinase inhibitors (Williams et al., 2012). Now a days, following chemotherapeutic agents are mostly used:

**a. Lestaurtinib**

This is also known as CEP-107 and is examined in persons that suffered from FLT3 gene transformation with intricate, declined or poor-risk AML (Smith et al., 2004). According to the prior study, this drug was effective on 60% patients with FLT3 mutations (Levis et al., 2006). It is a dual FLT3 inhibitor and has shown activity as monotherapy in AML (Knapper et al., 2006; Levis et al., 2011).

**b. Midostaurin**

Midostaurin (PKC412) is a semi-synthetic multitageted tyrosine kinase inhibitor, that revealed its activity as monotherapy in patients with FLT3 mutated gene and when it combines with standard drugs to give in newly diagnosed adults patients with AML, it shows complete response and survival rates (Fischer et al., 2010). It shows effectiveness in about 71% patients with FLT3 gene mutation (Stone et al., 2005).

**Table VI: Response criteria in AML (Cheson et al., 2003).**

<table>
<thead>
<tr>
<th>Category</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Complete remission (CR)*</td>
<td>Bone marrow blasts &lt;5%; absence of blasts with Auer rods, lack of extramedullary disorder, absolute neutrophil count &gt;100x10⁹/L, independence of red cell transfusion.</td>
</tr>
<tr>
<td>CR with incomplete recovery (CRi)†</td>
<td>All CR criteria except for residual neutropenia (&lt;1.0 x 10⁹/L) or thrombocytopenia (&lt;100 x 10⁹/L).</td>
</tr>
<tr>
<td>Morphologic leukemia-free state‡</td>
<td>Bone marrow blasts &lt; 5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required.</td>
</tr>
<tr>
<td>Partial remission (PR)</td>
<td>Relevant in the setting of phase 1 and 2 clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%.</td>
</tr>
<tr>
<td>Cytogenetic CR (CRc)§</td>
<td>Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow.</td>
</tr>
<tr>
<td>Molecular CR (CRm)</td>
<td>No standard definition; depends on molecular target.</td>
</tr>
<tr>
<td>Treatment failure</td>
<td></td>
</tr>
<tr>
<td>Resistant disease (RD)</td>
<td>Failure to achieve CR or CRi (general practice; phase 2/3 trials), or failure to achieve CR, CRi, or PR (phase 1 trials); only includes patients surviving ≥ 7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination.</td>
</tr>
<tr>
<td>Death in aplasia</td>
<td>Deaths occurring ≥7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia.</td>
</tr>
<tr>
<td>Death from indeterminate cause</td>
<td>Deaths occurring before completion of therapy, or &lt; 7 days following its completion; or deaths occurring ≥ 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available.</td>
</tr>
<tr>
<td>Relapse¶</td>
<td>Bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease.</td>
</tr>
</tbody>
</table>

* All criteria need to be satisfied; marrow assessment should be based on a count of 200 nucleated cells in an aspirate with spicules; if uncertainly, judge duplicate test after 5 to 7 days; flow cytometric evaluation may help to discriminate among determined leukemia and restore normal marrow; a marrow biopsy should be achieved in cases of dry tap, or if no spicules are acquired; no minimum extent of response obligatory.

† The criterion of CRi is of value in procedures using increased induction or double induction policy, in which hematologic improvement is not expected, although severe therapy will persist. In such procedures, CR can still not be attained in the course of the entire treatment plan. In these instances, the overall remission rate should include CR and CRi patients. Some patients may not achieve complete hematologic recovery upon longer observation times.

‡ This category may be useful in the clinical development of novel agents within phase 1 clinical trials, in which a transient morphologic leukemia-free state may be achieved at the time of early response assessment.

§ Four studies showed that failure to convert to a normal karyotype at the time of CR predicts inferior outcome (Freireich et al., 1992; Ballesien et al., 2009).
C. Sorafenib

Sorafenib (BAY 43-9006) is a drug with much of kinase activity that has an effect on platelet-derived growth factor (PDGF) and fibroblast growth factor receptor (FGFR). It is one of the most investigated FLT3 first generation inhibitors and has shown to specifically reduce the percentage of leukemia blasts in the peripheral blood (7.5% from 81%) and bone marrow (34% from 75.5%) of AML patients that have FLT3-ITD alteration but not in patients lacking this alteration (Zhang et al., 2008). It can also reveal activity in FLT3-ITD positive AML relapsing patients after allogenic stem-cell transplantation (Sharma et al., 2011).

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d. Sunitinib

Sunitinib (SU11248) is a small molecule inhibitor of RAF, vascular endothelial growth factor 2, c-KIT and FLT3 (Wilhelm et al., 2004). It was permitted as treatment agent in patients that suffered from kidney or gastrointestinal stromal cancers who were intolerant to imatinib (Fiedler et al., 2005, 2010) (Table VII).

Quizartinib (AC220) exhibit low nanomolar potency, good bioavailability and exceptional kinase selectivity and also a second generation FLT3 inhibitor (Zarrinkar et al., 2009). It shows a meaningful reduction in marrow blasts in both refractory and relapsed FLT3-ITD and AML patients (Cortes et al., 2011).

Besides these, other drugs that have shown preclinical activity and presently being investigated (Pemmaraju et al., 2011). However, sometimes a resistance occurs against AC220, which is because of the development of substitutional mutation in the activation loop of aspartic acid residue at position 835 (D835) (Sato et al., 2011).

AC220 is unique for FLT3 inhibitors and have high potency, favorable pharmacokinetic properties and excellent kinase selectivity (Zarrinkar et al., 2009). It is very active against FLT3 in elderly patients with either relapsed, refractory or untreated AML (Cortes et al., 2009). The clinical activity of AC220 is significant and causes a reduction in blast quantity and complete remission in several patients (Pemmaraju et al., 2011).

Table VII: Currently enduring clinical trials using fms-Like Tyrosine Kinase 3 receptor inhibitors for the treatment of acute myeloid leukemia (Pemmaraju et al., 2011).

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Patient Population</th>
<th>FLT3 Mutational Status</th>
<th>Combination</th>
<th>Recruitment Status</th>
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</thead>
<tbody>
<tr>
<td>Midostaurin</td>
<td>Newly investigated, aged &lt;60 yrs</td>
<td>Mutants only</td>
<td>Daunorubicin and cytarabine</td>
<td>Recruiting</td>
</tr>
<tr>
<td>AC220</td>
<td>Relapsed/refractory, aged ≥18 yrs</td>
<td>ITD mutants</td>
<td>NA</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Recently detected, ages 18-60 yrs</td>
<td>All</td>
<td>Standard chemotherapy</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Lestaurtinib</td>
<td>Relapsed, aged ≥18 yrs</td>
<td>Mutants only</td>
<td>Induction chemotherapy</td>
<td>Not recruiting</td>
</tr>
<tr>
<td>Midostaurin</td>
<td>Poor-risk, aged ≥60 y/aged &gt;70 yrs</td>
<td>All</td>
<td>Azacitidine</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Aged ≥60 yrs</td>
<td>All</td>
<td>Cytarabine</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Aged ≥60 yrs</td>
<td>Mutants only</td>
<td>Standard chemotherapy</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Midostaurin</td>
<td>Newly diagnosed, ages 18-60 yrs</td>
<td>All</td>
<td>Daunorubicin and cytarabine</td>
<td>Not recruiting</td>
</tr>
<tr>
<td>AC220</td>
<td>Relapsed/refractory, aged ≥18 yrs</td>
<td>All</td>
<td>NA</td>
<td>Not recruiting</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Relapsed/refractory, ages 2-20 yrs</td>
<td>All</td>
<td>NA</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Relapsed/refractory, aged ≥18 yrs</td>
<td>All</td>
<td>NA</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Relapsed/refractory, ages 0-31 yrs</td>
<td>All</td>
<td>Cytarabine and clofarabine</td>
<td>Recruiting</td>
</tr>
</tbody>
</table>

FLT3, fms-like tyrosine kinase 3 receptor; NCT, National Clinical Trials; ITD, internal tandem duplication; NA, not applicable; G-CSF, granulocyte colony-stimulating factor f. AC220.
Overall it shows CR and higher response rate in patients with FLT3-ITD mutation (56% and 28% respectively) as compared to those who do not have this mutation (Cortes et al., 2009).

g. AP24534

Ponatinib (AP24534) is a new multi targeted tyrosine kinase inhibitor (TKI) that is concerned as a predictive measure of other blood disorders including FLT3 (O’Hare et al., 2007). It results in inhibition of FLT3 signaling and induction of self-eating in FLT3-ITD affirmative AML cells with many primary blast cells (Gozgit et al., 2011)

Conclusions

- AML is a heterogeneous disease with marked differences in survival rates following chemotherapy based on age, blast cell morphology, cytogenetic abnormalities and gene mutations.

- The most important genetic abnormalities which occur in FT3 gene in AML patients show a poor prognosis and low survival rate. This also shows relapse during chemotherapeutic treatment. So there is a great need of molecular and cytogenetic improvements in the treatment of AML. Despite the fact that AML was the first human cancer genome to be sequenced and molecularly characterized, it is not properly treated yet due to the lack of targeted therapeutic options. Existing therapeutic approaches include the discovery and development of novel agents with unique structures conferring higher potency and selectivity toward FLT3 as a target.

Acknowledgement

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ROLE OF FLT3 GENE IN MYELOID LEUKEMIA PATIENTS


