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Review Article

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

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Abstract
Natural hematopoises 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).

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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

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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*., 2012). 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 cytrabine 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' lake 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.

Risk profile	(i) (Dohner *et al*., 2010)	(ii) (Patel *et al*., 2011)	(iii) (Grossmann *et al*., 2012)
Favorable	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 (*)	Favorable cytogenetics NPM1 mutation/FLT3-ITD- with IDH1 or IDH2 mutations	Very favorable: PML-RARA CEBPA double-mutation
			Favorable: RUNX1-RUNX1T1 CBFB-MYH11 NPM1 mutation/FLT3-TD-
Intermediate	Intermediate-I: NPM1 mutation/FLT3-ITD (*) NPM1 wt/FLT3-ITD + (*) NPM1 wt/FLT3-ITD- (*)	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	CEBPA single-mutation/FLT3-ITD + NPM1 mutation/FLT3-ITD + Wild-type cases
	Intermediate-II: t(9,11)(p22;q23); MLLT3-MLL Cytogenetic abnormalities not classified as favorable or adverse		
Unfavorable	inv(3)(q21q26.2)or t(3,3)(q21;q26.2); RPN1-EVI1 t(6,9)(p23;q34); DEK-NUP214 t(v;11)(v;q23); MLL rearranged − 5 or del(5q); -7; abnormality(17p); complex karyotype	Unfavorable cytogenetics FLT3-ITD+, CEBPA wt and any of these gene alterations: MLL-PTD, TET2, DNMT3A mutations or trisomy 8	Unfavorable: MLL-PTD and/or RUNX1 and/or ASXL1 mutation
			Very unfavorable: TP53 mutation

(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).

AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
Acute promyelocytic leukemia with t(15;17)(q22;q12); PML-RARA
AML with t(9 ;11)(p22;q23); MLLT3-MLL
AML with t(6;9)(p23;q34); DEK-NUP214
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
AML with mutated NPM1
AML with mutated CEBPA
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis
Myeloid leukemia associated with Down syndrome
Blastic plasmacytoid denderitic cell neoplasm

Table III: FAB Classification system of AML.

FAB subtype	**Name**	**Adult AML patients (%)**
M0	Undifferentiated acute myeloblastic leukemia	5
M1	Acute myeloblastic leukemia with minimal maturation	15
M2	Acute myeloblastic leukemia with maturation	25
M3	Acute promyelocytic leukemia	10
M4	Acute myelomonocytic leukemia	20
M4eos	Acute myelomonocytic leukemia with eosinophilia	5
M5	Acute monocytic leukemia	10
M6	Acute erythroid leukemia	5
M7	Acute megakaryocytic leukemia	5

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 conjugation 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).

Appearance of indicators for diagnoses	Prediction of acute myeloid leukemia (AML)*
Prrecursor stage HLA-DR	CD34, CD38, CD117,CD133
Granulocytic indicators cytoplasmic myeloperoxidase (cMPO)	CD13, CD15, CD16, CD33, CD65
Monocytic indicators	Nonspecific esterase (NSE), CD11c, CD14, CD64, Isozyme, CD4, CD11b, CD36, NG2 homologue‡.
Megakaryocytic markers	CD41 (Glycoprotein IIb/IIIa), CD61 (Glycoprotein IIIa), CD42 (Glycoprotein Ib).
Erythroid indicator	CD235a (Glycoprotein A)

*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-RARa-A and PML-RARa-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: Group1 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.

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

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 α(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 lymphohematopoitic 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, resultantly 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 (Kottaridis *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).

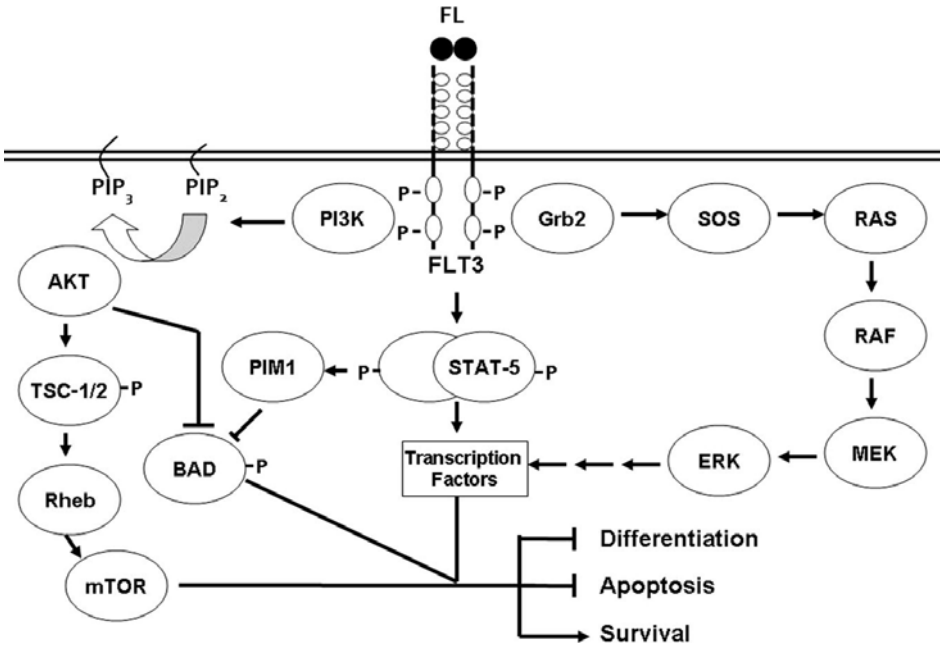

Figure 1: Basic diagram of stimulus downstream flow of FLT3 that are considered supportive to leukemia formation (Fathi and Chabner, 2011).

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.

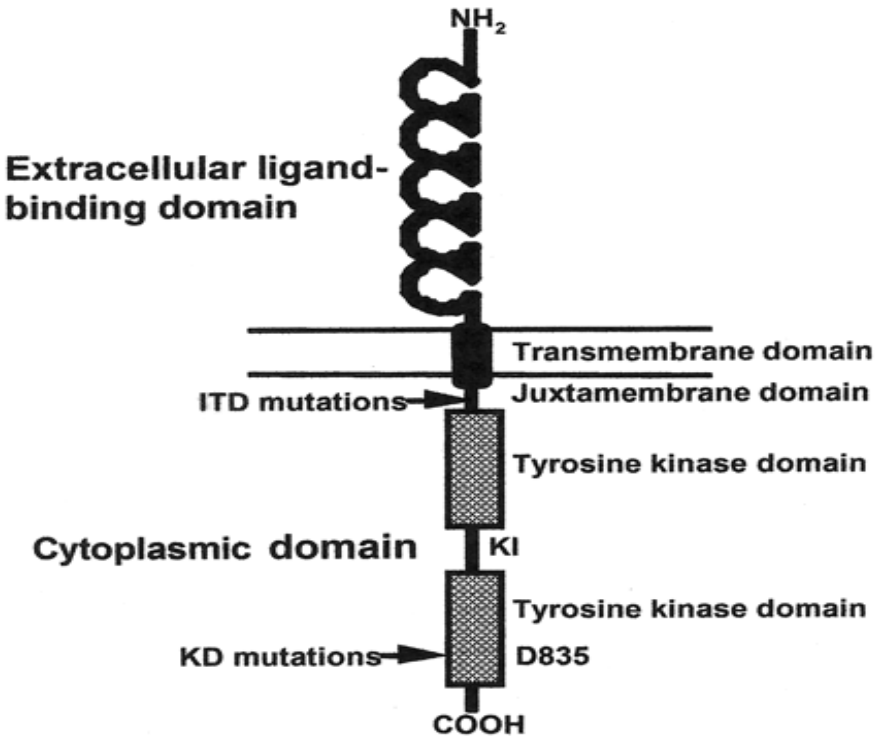

Figure 2: Diagrammatic representation of flt3 gene itd and kd domain (Small, 2006).

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 (Grundler *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 occured 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 (Lekstorm-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 have double-mutated 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 dehdrogenases 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 hydroxymethylecytosine, 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 methyletransferase and transcriptional co-activator that plays an important function in early progression and hematopoises (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*., 2011).

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 cytrabine 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 hematopoeitic 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 cytrabine 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 cytrabine (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 (Lestaurtinib), 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 histon 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).

Category	Definition
Complete remission (CR)*	Bone marrow blasts <5%; absence of blasts with Auer rods, lack of extramedullary disorder, absolute neutrophil count >100x109/L, independence of red cell transfusion.
CR with incomplete recovery (CRi) †	All CR criteria except for residual neutropenia (<1.0 × 109/L) or thrombocytopenia (<100 × 109/L).
Morphologic leukemia-free state‡	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required.
Partial remission (PR)	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%.
Cytogenetic CR (CRc)§	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.
Molecular CR (CRm)	No standard definition; depends on molecular target.
Treatment failure	
Resistant disease (RD)	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.
Death in aplasia	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.
Death from indeterminate cause	Deaths occurring before completion of therapy, or < 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.
Relapse¶	Bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease.

* 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).

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).

e. Quizartinib

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 meaningfull 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 resistence occurs against AC220, which is because of the developement 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 (Zarrinker *et al*., 2009). It is very active against FLT3 in elderly patients with either relapsed, refactory 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).

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

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.

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