

## MINI-REVIEW

# Molecular Involvement and Prognostic Importance of Fms-like Tyrosine Kinase 3 in Acute Myeloid Leukemia

Sadaf Shahab\*, Tahir S Shamsi, Nuzhat Ahmed

### Abstract

AML (Acute myeloid leukemia) is a form of blood cancer where growth of myeloid cells occurs in the bone marrow. The prognosis is poor in general for many reasons. One is the presence of leukaemia-specific recognition markers such as FLT3 (fms-like tyrosine kinase 3). Another name of FLT3 is stem cell tyrosine kinase-1 (STK1), which is known to take part in proliferation, differentiation and apoptosis of hematopoietic cells, usually being present on haemopoietic progenitor cells in the bone marrow. FLT3 act as an independent prognostic factor for AML. Although a vast literature is available about the association of FLT3 with AML there still is a need of a brief up to date overview which draw a clear picture about this association and their effect on overall survival.

**Keywords:** AML - FLT3 - ITDs - (TKD) - leukemia-specific recognition markers - prognostic marker

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### Introduction

Leukemia is a type of blood cancer where immature hematopoietic cells propagate in an abandoned manner. Bone marrow is the origin of Leukemia and there after it spreads rapidly anywhere (Islam, 1992). There are four main types of Leukemia, Acute Myeloid Leukemia (AML), Acute Lymphoblastic Leukemia (ALL), Chronic Myeloid Leukemia (CML) and Chronic Lymphocytic Leukemia (CLL). Acute Myeloid Leukemia (AML) is a blood cancer where abandoned growth of immature myeloid cells (myeloblasts, promyelocytes, monoblasts, erythroblasts, megakaryoblasts) in the bone marrow reduces the number of platelets, white blood cells and red blood cells.

AML is classified into four subtypes depending upon their stage of maturation and differentiation, which are: Acute Myeloid Leukemia with maturation, Acute Promyelocytic Leukemia (APL), Acute Erythroleukemia, Acute Myelomonocytic Leukemia, Acute Monocytic/Monoblastic Leukemia, Acute Megakaryoblastic Leukemia and Acute Myeloid Leukemia without maturation (Warner et al., 2005; Li and Amit, 2009). Acquired mutations in AML, interrupt the typical process of differentiation in the blood-forming cells, which results in the accumulation of large numbers of immature cells or myeloblasts. In case, erythroid lineage is affected, it can not perform the full functions of typical healthy red blood cells and creates anemic condition (Warner et al., 2005).

The molecular classification of AML was initiated and role of independent prognostic markers in AML

was started gaining popularity about two decades back (Bloomfield et al., 1984). The study of some of these markers has provided knowledge and understanding of disease pathogenesis and their biological and clinical outcomes. This approach has now been included into the classification of haemopoietic and lymphoid neoplasms of recent World Health Organization (WHO). The cytogenetic categories and risk groups are added in the 4<sup>th</sup> edition of the WHO guidelines. Patients are classified according to their cytogenetics into three different risk groups. This group include poor/worsen response group, favourable response group and standard/intermediate group (Elias et al., 2011).

The unplanned high dose chemo therapy is now restricted and molecular prognostics markers and cytogenetics become the main basis to develop a more risk free disease stratification plan. The main beneficiaries of this approach are inv(16), t(15;17) and t(8;21) (David et al., 2010)

Grimwade et al. (1998) reported that patients included in bad risk group disease have worsen or inferior survival rate because of partial/complete loss of genetic material. Such abnormalities create an unfavorable response to targeted therapy. Approximately 50% of patients have standard risk group; but their increased relapse rate is the main obstacle (David et al., 2010). Although some of these patients have abnormal karyotype, but about half of patients, have a normal karyotype (Richard et al., 2008; David et al., 2010). Therefore it is prerequisite to identify focal leukemic markers which help in deciding the route of treatment for the patients.

In this review we try to update the prognostic

involvement of FLT3 mutations on overall survival, remission and relapse.

## What We Already Know about Fms-like Tyrosine Kinase

Fetal liver tyrosine kinase-2 (FLK-2) or FLT3 (fms-like tyrosine kinase 3) or (STK1) (stem cell tyrosine kinase-1 is actually a member of the class III tyrosine-kinase receptor-family (RTK) which helps in apoptosis of hematopoietic cells and their proliferation (Yeo et al., 2010). It is the single most common mutated genes in AML and chiefly expressed by early myeloid and lymphoid progenitor cells (Rosnet et al., 1993), and also present on placenta, brain, cerebellum and gonads (Maroc et al., 1993; Frascella et al., 2006). The gene of FLT3 consists of 24 exons and is localized on chromosome 13q12 (Birg et al., 1994; Shurin et al., 1998; Abu et al., 2001). Approximately 5-15% of children and 25-35% of adults exhibit FLT3 mutation (Stirewalt and Radich, 2003; Schinichiro, 2011). A vast variety of literature considered FLT3 as bad prognostic marker (David et al., 2010; Patrick et al., 2011).

## What are the Biological Consequences of FLT3?

Internal tandem duplications (ITDs) is the first FLT3 mutations encoded by exons 14 and 15 which was identified unexpectedly (Nakao et al., 1996). Unpredictably lengthy segments of DNA were noticed in the PCR products of five AML patients. The isolation of genomic DNA from same patient, ruled out the alternate splicing. Additional studies confirmed that they all have duplicated sequences. These duplicated regions may contain insertion of additional nucleotides. Size and location of the duplicated region may vary person to person but always cause in frame mutation (Yokata et al., 1997).

## Frequency of FLT3/ITD in Patients and Healthy Individuals

The over expression of FLT3 mRNA is the main culprit in AML patients (David et al., 2010). FLT3/ITDs are the single most frequent mutation present in AML patients Schinichiro (2011). The over all frequency of ITD was 24% (Gale, 2003; Murphy et al., 2003) although few studies reported as high as 38 % and 44% (Govedarovic, 2011). Their prevalence in myelodysplastic syndromes (MDS) is 3% (Horiike et al., 1997) while occasional in patients of ALL (Nakao et al., 1996; Yokata et al., 1997). Although Yokata et al. (1997) has reported previously that FLT3/ITD is absent in chronic lymphoid leukaemia (CLL), non-Hodgkin's lymphoma and chronic myeloid leukaemia (CML), but Xu et al. (1999) has reported that FLT3/ITD seldom involved (2/84 ie 2.3%) in CML.

The frequency of FLT3/ITD mutations is low (0-7%) in patients of favorable group (Kottaridia et al., 2001; Frohling et al., 2002; Schnittger et al., 2002; Thiede et al., 2002). The FLT3/ITD is limited to exon 14 and 15. The FLT3/ITD is a mutation of somatic origin therefore it

is absent in normal individuals or in samples of patients which are in remission phase (Ishii et al., 1999; Kottaridis, 2001).

Many studies reported the presence of multiple or variable numbers of ITD mutations (2-5 additional bands) in same patient (Ishi et al., 1999; Kottaridis et al., 2001; Schih et al., 2002; Schnittger et al., 2002; Thiede et al., 2002). These mutations are independent as duplicated sequences are different. Some times, partial or complete absence of wild type allele is also reported in patient having normal karyotype (Kottaridis et al., 2001; Frohling et al., 2002; Schnittger et al., 2002; Thiede et al., 2002; Whitman et al., 2002).

## Origin of FLT3/ITD

A DNA-replication error might produce FLT3/ITD (Nakao et al., 1996). Up till now, no definite theory is reported about the origin of FLT3-ITD. Few reporters suggested Topoisomerase II, could cause breaks in the DNA (Libura et al., 2002) and this may result in decreased efficiency of the repair system (Zhong et al., 1999; Gaymes et al., 2002; Kiyoi et al., 2002). The JM domain elongation might contribute in constitutive activation and the mutant FLT3 receptor (Schinichiro et al., 2011).

## Mutations in the Second Tyrosine Kinase Domain (TKDs)

Yamamoto et al. (2001) and Abu-Duhier et al. (2001b) independently discovered point mutations in exon 20, in the TK domain. The overall frequency of TKD mutations reported is 7.1%. These mutations mostly codes for histidine and tyrosine. The insertion of few nucleotides or complete deletion of I836, have also been reported, but these mutations are always in frame.

Yamamoto et al. (2001) reported occasional cases in MDS is (3.4%) and 2.8% in ALL. The mutation causes constitutive tyrosine phosphorylation which result in gain of function (Yamamoto et al., 2001; Gale, 2003). The effect of TKD mutations are more or less similar to FLT3/ITD mutations which result in disturbance of auto inhibitory mechanism that prevent cells from uncontrolled signaling. More while few authors also reported the addition of 6 bp nucleotides which insert amino acids glycine and serine between codon 840 and 841 in 0.5% AML patients (Spiekermann et al., 2002; Gale 2003).

## Biological Features of FLT3/ITD Mutations in AML Patients

Many studies suggested that the activating mutations of receptor tyrosine kinases (RTKs) and over expression are chiefly involved in pathogenesis. (Gu et al., 2011). Approximately 25-30% of patients are ITD positive (Kottaridis et al., 2001; Schnittger et al., 2002; Thiede et al., 2002). ITD are always in frame mutations but may varies in length.

The frequency of FLT3/ITD mutations is low in patients having favorable cytogenetics i.e inv (16) (0-

**Table 1. Clinical and Pathological Characteristics of the AML Cases in this Study**

Study	No. of patients	Pediatric AML	Adult AML	Norm/Intr Cytogenetics (a)	8:21 or INV16	Poor risk Cyto (b)	Secondary AML (c)	Elderly AML (d)	Survival
Horiike et al	30						14.70% (5/34)		ND
Kiyoi et al	74								Decreased
Yamamoto et al	429		18.90% (81/429)	28.00% (23/82)	11.80% (4/34)	18.20% (2/11)			Decreased
Rombousts et al	75		23.70% (14/59)			0 (0/6)			Decreased
Abu-Duhier et al				22.70% (10/44)	5.80% (3/52)				Decreased
Stirewalt	140							33.60% (47/140)	No Effect
Kottaridis et al	854			36.80% (49/133)	34.20% (96/281)	8.30% (9/109)	3.40% (5/147)	27.40% (17/62)	Decreased
Whitman et al	82				28.00% (23/82)				No Effect
Iwai et al	94	5.30% (5/94)							Decreased
Xu et al	87	13.80% (12/87)							Decreased
Kondo et al	64	10.90% (7/64)							Decreased
Meshinchi et al	91	16.50% (15/91)							Decreased
Liang et al	80	11.30% (9/80)							No Effect -2
Thiede et al	979		22.20% (217/979)	29.70% (134/451)	4.50% (4/88)	1.70% (5/298)	8.80% (3/34)		Decreased
Schnittger et al	1003		23.30% (234/1003)	39.30% (149/379)	5.40% (6/11)	2.70% (3/110)	15.60% (12/77)		No Effect
Boissel et al	159		25.20% (40/159)	35.40% (28/79)	0 (0/23)				No Effect
Noguera et al									No Effect
Frohling et al	523		22.80% (119/523)						Decreased
Kainz et al	100			30.20% (16/53)	7.70% (2/26)				Decreased
Moreno et al	208				0% (0/14)				Decreased
Jilani et al	85		21.20% (18/85)						Decreased
Arrigoni et al	45	22.20% (10/45)							Decreased
Zwan	234	11.50% (27/234)							Decreased
Xu	103		23.20% (22/103)						RR is 66.8% -1
Natasa	113		17.70% (20/113)						Decreased
Wang	76		19.70% (15/76)						Decreased
Dohner	300		12.7% in NPM wt 19.7% in NPM mut						Decreased
Peerdeep	133		20%						No Effect
Rababab Aly	39								Decreased
Govedarovic and Marjanovic	103		44.7%						Decreased
Schlenk et al	531		31% (164/531)						Decreased

7%) and t(8;21) (5-9%) (Kottaridis et al., 2001; Frohling et al., 2002; Schnittger et al., 2002) and both of these translocations block differentiation (Deguchi et al., 2003), and wild type FLT3 gives survival benefit. Other subgroups comprises of 11q23 abnormalities (0-4%) and group of complex cytogenetics (2-3%), also have very low incidence of FLT3/ITD .

The incidence of FLT3/ITD and TKD mutations in the same patient is very low, 0-3% (Yamamoto et al., 2001; Frohling et al., 2002; Noguera et al., 2002; Palumbo et al., 2002) because of no survival advantage.

### Effect of ITD Mutations on Remission, Relapse and Overall Survival

About 50% of AML patients are cytogenetically normal (Katharina et al., 2011). The chromosomal abnormalities are absent in those patients but somatic genetic mutations are added in the spectrum of prognostic markers in AML patients more vigorous than ever examples are; c-KIT, and nucleophosmin member 1 (NPM1) and CCAAT enhancer binding protein alpha (CABP-a) (Richard et al., 2008; Farhad, 2010; James, 2010). Although the frequency of some mutation are very low ie 1.4% in case of KIT mutation (Kondo et al., 1999) and their clinical outcomes are not well studied. In case of conventional therapy about 60-80% of patients reached complete remission (CR) but relapse occur in majority of the cases (Kondo et al., 1999).

Cytogenetics is considered as most powerful prognostic marker uptill now (David et al., 2010) according to their karyotype (Kondo et al., 1999). Efforts are being made in recent years to identify new prognostic markers in order to envisage the clinical outcome in those patients (David et al., 2010).

FLT3/ITD is a crucial prognostic factor where cytogenetics is normal (David et al., 2010). Although, it produced no effect on complete remission (CR) but in children reduced CR has been reported (Kondo et al., 1999; Mesinchi et al., 2001; Zwaan et al., 2002). ITD is clearly associated with decreased disease-free survival (DFS), overall survival (OS) and increased relapse risk (RR), below 60 years of age (Kottaridis et al., 2001; Mesinchi et al., 2001; Frohling et al., 2002; Rosemary et al., 2008). Xu et al. (2000) reported very low frequency of ITD in children while Stirewalt et al, 2001 reported its non significant impact on clinical outcome in patients above 55 years of age. This group generally have overall unfavourable disease outcome (Schneider et al., 2011). Gregory et al. (2009) also reported that FLT/ITD is more frequent in adult patients than infantile AML patients. Many studies reported that patients having ITD frequently relapsed and their survival is shorter than patients having wild type FLT3 (Mesinchi et al., 2001; Schnittger et al., 2002; Rosemary et al., 2008).

Exhaustive literature reported the FLT3/ITD as an indicator of bad prognosis in less than 60 years of age (Kottaridis, 2001; Mesinchi et al., 2001; Frohling et al., 2002; David et al., 2010). It has been reported that overall survival and disease-free survival are significantly lower for ITD positive patients (Table 1). One important factor which should not be overlooked is the ratio between FLT3

wild type allele and FLT3 mutant allele. The FLT3-ITD load, loss of heterozygosity or loss of wild type allele as it usually result in lower overall survival and Disease free survival. (Schnittger et al., 2002; Schneider et al., 2011).

### Conclusion

Many studies have shown that ITD mutations have clear relationship with the prognosis and clinical response in AML. The data shown in Table1 clearly suggests that FLT3/ITD result in the overall poor prognosis. AML patients with FLT3/ITD mutation had an obviously increased expression level of FLT3/ITD upon relapsed proposed that FLT3/ITD might involved in constitutive stimulation and proliferation of residual blast cell. The identification of FLT3 mutations might be an important step for optimization of patient care. Because FLT3 ITD mutations portend a worsen prognosis, it has been proposed that patients who are FLT3/ITD positive may benefit from an allogeneic bone marrow transplantation instead of aggressive chemotherapy.

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