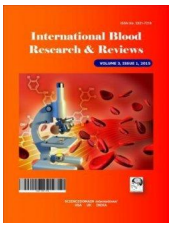

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FLT3 Mutations: Significance in Paediatric AML

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Author's contribution

Author SMA the sole author of this paper, takes all the credential for writing and reviewing the manuscript.

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ABSTRACT

Patients with acute myeloid leukaemia (AML) who have FMS-like tyrosine kinase 3 (FLT3) mutations are a concern for haematologists. Many studies indicate that these patients have poor prognosis. Due to the dearth of research in this area, the role of allogeneic transplantation as a therapy is still controversial. But the available limited data suggest that transplantation in first remission is possibly the first choice. As FTL3 mutations in AML result in poor patient outcomes, different FLT3 inhibitors are used as specific targeted therapy.

Keywords: Myeloid leukaemia; FMS-like tyrosine kinase 3; allogeneic transplantation; haematopoiesis.

1. INTRODUCTION

Acute myeloid leukaemia (AML) is a fatal disease characterised by clonal expansion of undifferentiated myeloid precursors. It results in impaired haematopoiesis and bone marrow (BM) failure [1]. Although many of the younger AML patients have a favourable response to induction chemotherapy and allogeneic transplantation, there is a higher relapse rate. Thus, chemotherapy and allogeneic transplantation in AML are associated with treatment failure [2]. Moreover, half of the patients appear to be cytogenetically normal

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(CN) for the disease with no detectable chromosomal aberrations.

Many types of mutations can result in molecular abnormality in AML patients [3-6]. The most common type of mutations involves the FLT3 gene, followed by the mixed lineage leukaemia (MLL) gene [7,8]. Among the FLT3 mutations, FLT3/ITD is the most clinically relevant type with poor prognostic outcomes [9].

The purpose of this review is to present the currently available evidence pertaining to the significance of FLT3 mutations in paediatric AML patients. This review also provides information on novel small-molecule FLT3 tyrosine kinase inhibitors (TKI) which are in the development phase.

2. FLT3 RECEPTOR

The most common and clinically relevant molecular abnormality in AML is the mutations in the FLT3 gene [9]. This gene codes for the transmembrane tyrosine kinase receptor, which belongs to the tyrosine kinase family. This receptor is present in the leukaemic blast cells in most AML patients. The membrane-bound receptor is expressed in various progenitor cells and controls the division and differentiation of hematopoietic cells [10]. FLT3 receptor is composed of juxtamembrane dimerization domain, transmembrane domain, extracellular domain and intracellular kinase domain (Fig.1).

Initially, FLT3 is present in a monomeric, unphosphorylated state. As soon as the receptor binds with the FLT3 ligand (FL), the receptor goes through homodimerization and undergoes conformational changes. Phosphorylation of various sites in the intracellular kinase domain occurs due to FLT3 dimerization. This activates the signalling cascade of MAP kinase, STAT, and AKT/PI3. Thus, the FLT3 receptor regulates the cell proliferation, differentiation and inhibition of apoptosis [11]. In the process of normal haematopoiesis, the expression of FLT3 receptor promotes a perfect hierarchy in the haematopoietic stem and early progenitor compartment [12].

As previously mentioned, FLT3 mutations are the most common and clinically relevant mutations in AML, contributing to around one-third of the mutations. The next common mutations are those involving the MLL gene. Even these mutations have a negative prognostic value. There are two common variants of FLT3 mutations. The first type is the internal tandem duplications on exon 14 of FLT3 receptor (FLT3/ITD). This interferes with the function of the juxtamembrane domain of the receptor and results in ligand-independent stimulation of FLT3 receptor, which results in the proliferation of leukaemic cells independent of the growth factors. The second type of mutations is the mutations in the tyrosine kinase domain (FLT3/TKD mutations). These are the point mutations occurring in the activation loop with single amino acid substitutions [13]. Among these two mutations, FLT3/ITD mutations are mostly seen in AML patients. FLT3/ITD mutation leaves FLT3 kinase constitutively active [14], and it is observed in around 30-40% of AML patients. Numerous studies have recognized that these mutations cause higher relapse rates and shorter overall and disease-free survival [15-17]. Furthermore, the expressions of FLT3 protein and RNA are up-regulated in AML cells, compared with normal BM cells. Over-expression of this gene and the length and position of insertion of ITD are associated with lower survival rates. Allogeneic transplantation in these patients was associated with favourable patient outcomes like lower relapse rates and improved overall survival [18,19]. BM and peripheral blood

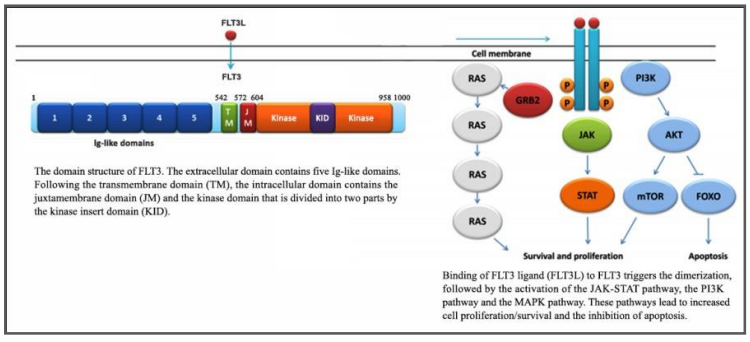

Fig. 1. FLT3 receptor structure and molecular cascade

are used to detect FLT3/ITD mutation. High molecular weight genetic DNAs were extracted from the AML cells using phenol-chloroform method. Using polymer chain reaction (PCR) method, the DNA was amplified for FLT3 gene at exons 14 and 15 with forwarding primer 5' GCAATTTAGGTATGAAAGCCAGC 3' and reverse primer 5' CTTTCAGCATTTTGACGGCAACC 3'. Several methods were used over a period of time for the detection of FLT3/ITD mutation in AML patients.

3. FLT3/ITD MUTATIONS

A reverse transcriptase polymerase chain reaction (RT-PCR) assay was performed to detect the expression of FLT3 in the samples of primary leukaemia. The FLT3/ITD mutations were discovered for the first time during this process, and a molecular abnormality was noted in the FLT3 transcripts from AML patients [14]. FLT3/ITD mutations in patients with AML were associated with higher mean white blood cell (WBC) count, higher relapse rates and shorter overall and disease-free survival when compared with AML patients without FLT/ITD mutations. It was also observed that FLT3/ITD mutations are responsible for the emergence of drug resistance [20-22].

4. FREQUENCY OF FLT3/ITD MUTATIONS IN AML

Initially, it was thought that the FLT3/ITD mutations occur only in AML patients. However, recently, it is observed that these mutations are also a common variant in MLL. The assessment of the frequency of these mutations in AML patients depends on various factors. These mutations vary significantly in different subtypes of AML, which are classified by cytogenetic aberrations. The frequency of these mutations might be overestimated as the study samples mostly consist of a small cohort of hospitals or clinics [13]. Other factors that may influence the approximation of its occurrence are the methods and samples used to determine these mutations [13].

5. CHARACTERISTICS OF FLT3/ITD MUTATIONS IN PAEDIATRIC AML PATIENTS

5.1 Leukocytosis

Leukocytosis is considered as one of the poor prognostic factors in paediatric AML. FLT3/ITD mutations are associated with significantly higher WBC counts than the wild-type FLT3 [13].

5.2 Age

There is no significant association of FLT3 mutations with age in adults [13]. However, all of the studies, except two mentioned in Table 1, observed that FLT3/ITD mutations were less common in paediatric patients who were less than 10 years of age (Table 1). About two-thirds of the paediatric patients in these five studies mentioned in Table 1 were found to be more than 10 years old. Thus, the frequency of these mutations is much higher in children above 10 years than in the younger children. Genomic instability was considered as the possible mechanism for this difference [27].

5.3 FAB Subtypes

Many studies reported that all FAB subtypes are equally associated with FLT3 ITD mutations. However, some studies noted an increased prevalence of this mutation in M3 (Acute promyelocytic leukaemia (APL), M5 (Acute myelomonocytic leukaemia) and M5b (Acute monocytic leukaemia) subtypes [27-29]. Numerous groups around the world have now confirmed and extended these findings in both adult and paediatric AML. According to a study conducted by the National Cancer Institute, AML is an important example of a cancer type that establishes different genomic signatures between paediatric and adult patients despite phenotypic similarities. The study found declining incidence in AML over the first decade of life beginning at its highest rate (infant) to its lowest rate (9 years of age). However, this trend starts to reverse by the age of 10 years, following a slow increase in incidence up to the age of 40 years. This is followed by two successive bursts in AML incidences in older populations up to the highest recorded levels at age 84. Since severe variations in the frequency of mutation were observed between age groups, there is a need for targeted therapies based on these differences [24,30].

5.4 Survival Rate

The paediatric studies mentioned in Table 1 were analysed for the survival rate. Only 11 children survived out of 58 patients with FLT3/ITD mutations. The survival rate was found to be significantly less in patients with FLT3/ITD

mutations. Even though the frequency of these mutations is lower in paediatric patients than adults, the survival rate statistics seems to be alarming, requiring an equal attention to paediatric AML [27-29].

Table 1. Individual FLT3/ITD studies in paediatric groups with AML adapted from levis & small [13]

Study	Paediatric AML
Whitman et al (82 patients) [23]	5.3% (5/94)
Iwal et al (94 patients) [24]	13.8% (12/87)
Xu et al (87 patients) [25]	10.9% (7/64)
Kondo et al (64 patients) [26]	16.5% (15/91)
Meshinchi et al (91 patients) [27]	11.3% (9/80)

6. FLT3 INHIBITORS

Poor prognostic outcomes due to FLT3 mutations in AML patients have added to the credentials of this receptor as a treatment target. These small-molecule FLT3 inhibitors were proven to be cytotoxic to leukaemic cells as they inhibit the proliferation of these cells, causing cell cycle arrest and apoptosis [9]. These molecules belong to different chemical groups and vary significantly in their potency and selectivity. Numerous TKIs target FLT3 receptor, including sorafenib [31-34]. These drugs have shown promising results in AML patients. However, they have some limitations due to their varying responses in different patient groups and the emergence of resistance mutations in FLT3/ITD [35-37].

Quizartinib was shown to be considerably more potent than other TKI inhibitors [38,39]. In 2012, a phase 2 trial was conducted with Quizartinib in relapsed or refractory FLT3/ITD AML patients [40,41]. This drug effectively reduced the peripheral and BM blasts by apoptosis and terminal differentiation, respectively [42]. This resulted in long-term survival of one-third of the study patients by creating a favourable condition for undergoing allogeneic transplantation.

Some multikinase inhibitors like sorafenib and sunitinib, which are approved for other neoplasms, are shown to have inhibitory potential against FLT3. A study published in 2012 conducted a multicentric trial in relapsed AML patients with Sorafenib. In this study, about one-third of the patients had haematological remission. Another third had BM and complete remission. This drug was shown to produce sustained remissions in the patients who have relapsed after allogeneic transplantation. These drugs are prescribed for off-label use in AML patients by many clinicians [43,30].

FLT3 inhibitors have varied limitations, including the toxicity due to off-target effect leading to gastrointestinal intolerance, prolonged cytopenias and hand-foot syndrome. Another major clinical problem with the FLT3 inhibitor is the induction of acquired resistance. Hence, optimized target therapy and treatment option and strategy need to be prepared by the physician to overcome the challenges in different clinical settings.

7. CONCLUSION

AML is a fatal disease without the use of aggressive chemotherapy treatments. FTL3/ITD mutation in AML is the most common abnormality resulting in poor patient outcomes. These mutations occur in about one-third of patients. Therefore, it is important to screen all AML patients for these mutations as soon as the diagnosis is made or when AML is relapsed. The existing data suggest that FLT3 mutations are not enough for leukaemic transformation, and it involves other cooperating genomic modifications for the development of leukaemic phenotype. The best therapeutic approach for these patients is allogeneic transplantations followed by maintenance therapy with the effective TKI inhibitors like Quizartinib and Sorafenib. In a study, Quizartinib, a small-molecule receptor TKI, was reported to augment the survival of AML patient compared to control]. Thus, the addition of these FLT3 TKIs in the treatment algorithm of AML patients with FLT3 activating mutations will improve patient survival and their quality of life. Further large-scale clinical trials are needed to provide concrete clinical evidence for these conclusions.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Parikh S, Jabbour E, Koller C. Adult acute myeloid leukemia adult acute myeloid leukemia: Introduction epidemiology, etiology, and risk factors. MD Anderson Manual of Medical Oncology. 2014;1–25.
2. Dohner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med. 2015;373:1136–1152.
3. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368:2059–2074.
4. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The origin and evoluation of mutations in acute myeloid leukemia. Cell. 2012;150: 264–278.
5. Walter MJ, Shen D, Ding L, Shao J, Koboldt DC, Chen K, et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med. 2012;366: 1090–1098.
6. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506:328–333.
7. Dohner K, Dohner H. Molecular characterization of acute myeloid leukemia. Haematologica. 2008;93:976–982.
8. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 2005;352:256–266.
9. Levis M, Small D. FLT3: It does matter in leukemia. Leukemia. 2003;17:1738–1752.
10. Flip AA, Libura M, Giebel S, Haus O. Genetic mechanisms and molecular markers of neoplastic transformation in acute myeloid leukemia. In Witt M, Dawidowska M, Szczepanski T. Molecular aspects of hematologic malignancies, Berlin, Germany: Springer Berlin Heidelberg. 2012:29–54.
11. Meshinchi S, Appelbaum FR. Structural and functional alterations of FLT3 in acute myeloid leukemia. Clin Cancer Res. 2009;15:4263–4269.
12. Volpe G, Clarke M, Garcia P, Walton DS, Vegiopoulos A, Pozzo WD, et al. Regulation of the Flt3 gene in haematopoietic stem and early progenitor cells. PLoS One. 2015;10:e0138257.
13. Levis M. FLT3 mutations in acute myeloid leukemia: What is the best approach in 2013? American Society of Hematology 2013;2013:220–226.
14. Nakao M, Yokota S, Iwai T, Kaneko H, Horiike S, Kashima K, et al. Internal tandem duplication of the flt3 gee found in acute myeloid leukemia. Leukemia. 1996; 10:1911–1918.
15. Bienz M, Ludwig M, Leibundgut EO, Mueller B, Ratschiller D, Solenthaler M, et al . Risk assessment in patients with acute myeloid leukemia and a normal karyotype. Clin Cancer Res. 2005;11:1416–1424.
16. Kiyoi H, Naoe T, Nakano Y, Yokota S, Minami S, Miyawaki S, et al. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood. 1991;93:3074–3080.
17. Thiede C, Steudel C, Mohr B, Schaich M, Schäkel U, Platzbecker U, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: Association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002;99:4326–4335.
18. Bonardi F, Fusetti F, Deelen P, van Gosliga D, Vellenga E, Schuringa JJ. A proteomics and transcriptomics approach to identify leukemic stem cell (LSC) markers. Mol Cell Proteomics. 2013;12: 626–637.
19. Ozeki K, Kiyoi H, Hirose Y, Iwai M, Ninomiya M, Kodera Y. Biologic and clinical significance of the FLT3 transcript level in acute myeloid leukemia. Blood. 2004;103:1901–1908.
20. Yokota S, Kiyoi H, Nakao M, Iwai T, Misawa S, Okuda T, et al. Internal tandem duplication of the FLT3 gene is preferentially seen in acute myeloid leukemia and myelodysplastic syndrome

among various hematological malignancies. A study on a large series of patients and cell lines. Leukemia. 1997; 11:1605–1609.

21. Levis M, Allebach J, Tse KF, Zheng R, Baldwin BR, Smith BD, et al. A FLT3-targeted tyrosine kinase inhibitor is cytotoxic to leukemia cells in vitro and in vivo. Blood. 2002;99:3885–3891.
22. Quentmeier H, Reinhardt J, Zaborski M, Drexler HG. FLT3 mutations in acute myeloid leukemia cell lines. Leukemia. 2003;17:120–124.
23. Whitman SP, Archer KJ, Feng L, Baldus C, Becknell B, Carlson BD, et al. Absence of the wild-type allele predicts poor prognosis in adult de novo acute myeloid leukemia with normal cytogenetics and the internal tandem duplication of FLT3: A cancer and leukemia group B study. Cancer Res. 2001;61:7233–7239.
24. Iwai T, Yokota S, Nakao M, Okamoto T, Taniwaki M, Onodera N, et al. Internal tandem duplication of the FLT3 gene and clinical evaluation in childhood acute myeloid leukemia. Leukemia. 1999;13: 38–43.
25. Xu F, Taki T, Yang HW, Hanada R, Hongo T, Ohnishi H, et al. Tandem duplication of the FLT3 gene is found in acute lymphoblastic leukaemia as well as acute myeloid leukaemia but not in myelodysplastic syndrome or juvenile chronic myelogenous leukaemia in children. Br J Haematol. 1991;105: 155–162.
26. Kondo M, Horibe K, Takahashi Y, Matsumoto K, Fukuda M, Inaba J, et al. Prognostic value of internal tandem duplication of the FLT3 gene in childhood acute myelogenous leukemia. Med Pediatr Oncol. 1999;33:525–529.
27. Meshinchi S, Woods MG, Stirewalt DL, Sweetser DA, Buckley JD, Tjoa TK, et al. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood. 2001; 97:89–94.
28. Schnittger S, Schoch C, Dugas M, Kern W, Staib P, Wuchter C, et al. Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood. 2002;100:59–66.
29. Haferlach T, Schoch C, Schnittger S, Kern W, Loffler H, Hiddemann W. Distinct genetic patterns can be identified in acute monoblastic and acute monocytic leukaemia (FAB AML M5a and M5b): A study of 124 patients. Br J Haematol. 2002;118:426–431.
30. National Cancer Institute. Childhood and Adult Acute Myeloid Leukemia: Genetically Distinct Diseases. Available:https://ocg.cancer.gov/e-newsletter-issue/issue-10/childhood-and-adult-acute-myeloid-leukemia-genetically-distinct-diseases.
31. Auclair D, Miller D, Yatsula V, Pickett W, Carter C, Chang Y, et al. Antitumor activity of sorafenib in FLT3-deriven leukemic cells. Leukemia. 2007;21:439–445.
32. Fathi AT, Chabner BA. FLT3 inhibition as therapy in acute myeloid leukemia: a record of trials and tribulations. Oncologist. 2011;16:1162–1174.
33. Levis M, Ravandi F, Wang E, Baer MR, Perl A, Coutre S, et al. Results from a randomized trail of salvage chemotherapy followed by lestaurtinib for patients with FLT3 mutant AML in first relapse. Blood. 2001;117:3294–3301.
34. Fischer T1, Stone RM, Deangelo DJ, Galinsky I, Estey E, Lanza C, et al. Phase IIB trail of oral Midostaurin (PKC412), the FMS-like tyrosine kinase 3 receptor (FLT3) and multi-targeted kinase inhibitor, in patients with acute myeloid leukemia and high-risk myelodysplastic syndrome with either with-type or mutated FLT3. J Clin Oncol. 2010;28:4339–4345.
35. Levis M, Brown P, Smith BD, Stine A, Pham R, Stone R, et al. Plasma inhibitory activity (PIA): a pharmacodynamic assay reveals insights into the basis for cytotoxic response to FLT3 inhibitors. Blood. 2006;108:3477–3483.
36. Weisberg E, Sattler M, Ray A, Griffin JD. Drug resistance in mutatnt FLT3-positive AML. Oncogene. 2010;29:5120–5134.
37. Cools J, Mentens N, Furet P, Fabbro D, Clark JJ, Griffin JD, et al. Prediction of resistance to small molecule FLT3 inhibitors: impilcations for molecularly targeted therapy of acute leukemia. Cancer Res. 2004;64:6385–6389.
38. Zarrinkar PP, Gunawardane RN, Cramer MD, Gardner MF, Brigham D, Belli B, et al.

AC220 is a uniquely potent and selective inhibitor if FLT3 for the treatment of acute myeloid leukemia (AML). Blood. 2009;114: 2984–2992.

39. Cortes JE, Kantarjian H, Foran JM, Ghirdaladze D, Zodelava M, Borthakur G, et al. Phase I study of quizartinib administered daily to patients with relapsed or refractory acute myeloid leukemia irrespective of FMS-like tyrosine kinase 3-internal tandem duplication status. J Clin Oncol . 2013;31:3681–3687.
40. Levis MJ, Perl A, Dombret H, et al. Final results of a phase 2 open-label, monotherapy efficacy and safety study of quizartinib (AC220) in patients with FLT30ITD positive or negative relapsed/refractory acute myeloid leukemia after second-line chemotherapy or hematopoietic stem cell (abstra. Blood (ASH annual Meeting Avstracts). 2012; 120:673.
41. Cortes JE, Perl AE, Dombert H, Kayser S, Steffen B, Rousselot P, et al. Final results of a phase 2 open-label, monotherapy efficacy and safety study of quizartinib (AC220) in patients 60 years of age with FLT3-ITD positive or negative relapsed/refractory acute myeloid leukemia (abstract). Blood (ASH Annual Meeting Abstracts). 2012;120:48.
42. Sexauer A, Perl A, Yang X, Borowitz M, Gocke C, Rajkhowa T, et al. Terminal myeloid differentiation in vivo is induced by FLT3 inhibition in FLT3/ITD AML. Blood. 2012;120:4205–4214.
43. Metzelder SK, Schroeder T, Finck A, Scholl S, Fey M, Götze K, et al. High activity of sorafenib in FLT3-ITD-positive acute myeloid leukemia synergizes with allo-immune effects to induce sustained responses. Leukemia. 2012;26:2353–2359.

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