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Håkon Reikvam

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SPECIAL REPORT

Revisiting the prognostic role of *FLT3* mutations in acute myelogenous leukemia

Håkon Reikvam [a,b]

[a]Department of Clinical Science, University of Bergen, Bergen, Norway; [b]Department of Medicine, Haukeland University Hospital, Bergen, Norway

ABSTRACT

Introduction: Approximately one-third of patients with acute myelogenous leukemia (AML) harbor mutations in the fms-like tyrosine kinase 3 (*FLT3*) gene. The features regarding prognostic impact of *FLT3* mutated AML have been widely investigated and debated in the last decades, and the significance of this mutation is constantly evolving.

Areas covered: In this article, the significance of the *FLT3* mutation and various aspects of this mutation are discussed in the light of new understanding and research progress in past years. The recently updated European Leukemia Net (ELN) guidelines are reviewed and discussed, special emphasis given to the the improvement in therapeutic approaches for *FLT3* mutated AML.

Expert opinion: Aspects related to *FLT3* mutated AML include the type of mutation in addition to *FLT3*-internal tandem duplication (ITD) length, location, and allelic ratio. Furthermore, the coexistence of cytogenetic variants and molecular genetic mutations utterly complicate the evaluation of the prognostic impact. In addition, introduction of FLT3 inhibitors and establishment of measurable residual disease (MRD) monitoring have entered the treatment and evaluation armamentarium in the handling of AML patients, resulting in improved prognosis for these patients. However, future research to optimize the treatment of *FLT3* mutated AML is highly desired.

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Acute myelogenous leukemia; *FLT3*; targeted therapy; stem cell transplantation; prognosis

1. Introduction

Acute myelogenous leukemia (AML) is an aggressive malignancy characterized by the proliferation of immature myeloid leukemic blasts [1]. AML is a heterogenous disease, encompassing a wide range of chromosomal, molecular genetics, epigenetic, and biochemical alterations [1], which ultimately converge at the level of protein function and cell signaling pathways. In the most recent decade, the rapid improvement and widespread availability of genomic technology has allowed for high-fidelity sequencing of the leukemic clones [2]. Based on current data, on average, each AML patient genome harbors 13 mutations; however, some of these are recurrently mutated genes [3]. Among these recurrent genes is the FMS-like tyrosine kinase 3 *(FLT3*) gene, which is found to be mutated in approximately 25–30% of AML patients [4–6] and up to 40% in the group of cytogenetically normal AML [7]. Since the initial discovery of this mutation in AML over 25 years ago, its impact on treatment and prognosis has been extensively studied and debated [8–10]. Furthermore, as inhibitors of the FLT3 protein have been developed, the potential of targeting this oncogenic kinase has been exploited, leading to an evolving aspect of the prognostic impact of *FLT3* mutation in AML [8–11]. In the present, the history and current features of *FLT3* mutated AML are discussed, specifically emphasizing the prognostic impact and therapeutic implication it currently has in AML.

2. *FLT3* mutation biology

FLT3 is a receptor-type tyrosine kinase expressed on the cell surface of hematopoietic cells and is involved in cell differentiation and proliferation. It was first discovered and described in 1991 and belongs to the type III receptor tyrosine kinase class expressed by hematopoietic stem cells [12]. The receptor is encoded by the *FLT3* gene located on chromosome 13q12, and mutations in this gene in association to AML was first detected in 1996 [13]. Mutation could be of two major types: the *FLT3* internal tandem duplication (*FLT3*-ITD) in the juxta membrane domain (JMD) of the protein or a point mutation in the *FLT3* tyrosine kinase domain (*FLT3*-TKD). The *FLT3*-ITD mutation is formed by duplication of a fragment of the coding sequence within the intracellular region. Both the size of the ITD and the exact location within the gene varies, resulting in a functional kinase domain without the JMD, leading to autophosphorylation and activation signal transduction of the malignant cells. In contrast, the *FLT3*-TKD mutation results from missense point mutation within the activation loop in the TKD, with prediction for residue D835. This also leads to autophosphorylation and malignant cell growth. The ITD mutation occurs in approximately 20–25% of adult AML patients, while the TKD mutation occurs in only approximately 5–10% of the patients [4–7,14,15]. The *FLT3* mutation is rare in childhood AML; approximately only 10–15% of AML patients <18 years harbor this mutation

CONTACT Håkon Reikvam Hakon.Reikvam@uib.no Department of Clinical Science, University of Bergen, Bergen, Norway

Article highlights

- The *FLT3* gene is frequently mutated in acute myelogenous leukemia.
- The mutation has traditionally been considered to be associated with an inferior prognosis.
- Various aspects of the characteristics of *FLT3* mutations appear to influence prognosis, as well as coexisting genetic alterations
- Improved diagnostics and better treatment have, however, improved treatment results.
- There is still uncertainty regarding challenges for best risk classification and optimal therapeutic approaches for these patients.

[16,17]. The incidence is increasing with increasing age; however, in the oldest patient's cohort (>60 years), the mutation again seems to be less present [18,19]. The latter is probably related to increasing incidence of secondary or therapy related AML in the older patient cohort; there the mutation is more rarely seen in contrast to *de novo* AML [20–22]. The *FLT3* mutation is associated with leukocytosis and increased blast count [14,23]. Furthermore, the mutation is also associated with other recurrent mutation, and it specifically seems to co-occur with the mutation in the nucleophosmin 1 *(NPM1)* gene in approximately 50–60% of cases [7,8,24,25]. Also, other mutations and cytogenetic alterations can coexist with the *FLT3* mutation. Finally, the sizes of the ITD, the localization of the ITD, and the mutation allelic ratio, all show considerable variation between patients. Taken together, these observations indicate that *FLT3* mutated AML is a considerable heterogenetic disease ethnicity (Table 1).

3. Evolving role of the prognostic impact of *FLT3* mutation in AML

3.1. Prognostic impact of type of *FLT3* mutation

The *FLT3*-ITD mutation is the most common mutation in AML, and the prognostic impact of this mutation has been extensively evaluated and is probably dependent on several factors including the length of the ITD, the site of the ITD within the genome, the allelic ratio, and concomitant cytogenetic aberrations and molecular mutations (Table 1). On the other side, *FLT3*-TKD mutations have also been evaluated as a prognostic factor in AML; some reports have shown that *FLT3*-TKD mutations are associated with shorter remission and overall survival, while others have shown that *FLT3*-TKD mutations do not affect prognosis or even display a favorable prognosis [15,26–28]. These differences in results may be due to differences in patient backgrounds and treatment methods. Furthermore, it is believed that *FLT3*-TKD mutations are probably only rare drivers of the disease, and hence, their main contribution in the leukemic pathogenesis is probably limited.

3.2. Prognostic impact of *FLT3*-ITD allelic ratio

In 2014, the German-Austrian AML study group published a report indicating that high *FLT3*-ITD allelic ratio indicate mutated alleles compared to wild type alleles, defined by a cutoff ≥0.51 that was associated with unfavorable relapse-free and overall survival [29]. In contrast, after allogeneic hematopoietic stem cell transplantation (allo-HSCT), outcome was significantly improved in patients with a high allelic ratio, while on the other side, no benefit was seen in patients with a low allelic ratio [29]. After this study, the European

Table 1. FLT3 mutations features and association with prognosis in AML.

FEATURES	COMMENTS
TYPE of mutaion (ITD/TKD)	ITD mutations has traditionally been associated with adverse outcome, while the TKD mutations probably has less prognostic impact
ITD length	Patients with longer ITDs (>48 bps) seem to have more chemoresistance disease
Allelic ratio	High *FLT3*-ITD allelic ratio has been associated with an unfavorable prognosis, although methodical standardization has been an obstacle
Insertion site	Studies have demonstrated that ITDs outside the JMD have been associate with resistance to chemotherapy and inferior outcome
Presence of *NPM1* mutation	Coexistent of *NPM1* mutation in *FLT3* mutated cases have been of conflicting results, although MRD monitoring by PCR is currently preferable for reprognostication
Prescence of other mutations	Cooccurrence of *WT1*, *RUNX1*, and *ASXL1* mutations have been linked to inferior outcome
Presecnce of cytognetic alteations	Favorable and adverse cytogenetic prognostic alterations are mainly not affected by *FLT3* mutations which has strongest impact in normal karyotype AML

Table 2. The evolving role of FLT3 and NPM1 mutation regarding the ELN guidelines published in 2010, 2017, and 2022.

	ELN 2010 [8]	ELN 2017 [9]	ELN 2022 [10]
Favorable	*FLT3*wt-*NPM1*mut	*FLT3*wt-*NPM1*mut *FLT3*-ITD$^{low\ §}$-*NPM1*mut	*FLT3*wt-*NPM1*mut†¶
Intermediate*	*FLT3*-ITD-*NPM1*mut *FLT3*-ITD-*NPM1*wt *FLT3*wt-*NPM1*wt	*FLT3*-ITD$^{high\ §}$-*NPM1*mut *FLT3*-ITD$^{low\ §}$-*NPM1*wt	*FLT3*-ITD-*NPM1*mut†¶ *FLT3*-ITD-*NPM1*wt
Adverse		*FLT3*-ITD$^{high\ §}$-*NPM1*wt	

The table demonstrates the alterations in the recommendation for the prognostic impact for *FLT3* mutation during the previously published recommendation from the ELN.
*Classified as intermediate-I in the ELN 2010 classification.
§Low, low allelic ratio (<0.5); high, high allelic ratio (≥0.5).
¶AML with *NPM1* mutation and adverse-risk cytogenetic abnormalities are categorized as adverse-risk.
†Initial risk assignment may be change during the treatment course based on the results from analyses of measurable residual disease.

LeukemiaNet (ELN) incorporated allelic ratio in their 2017 revised version of their AML guidelines [9]; there patients with a high *FLT3*-ITD allelic ratio were classified as intermediate or unfavorable risk, in the presence or absence of the *NPM1* mutation, respectively (Table 2). In contrast, recent studies have failed to demonstrate the same prognostic impact of *FLT3*-ITD allelic ratio [30,31]. In addition, the lack of laboratory standardization, an ongoing debate regarding the definition of the cutoff between a high and low allelic ratio, and the likely benefits of introducing FLT3 inhibitors into induction therapy, lead to the exclusion of the possible prognostic impact of *FLT3*-ITD allelic ratio in the updated ELN 2022 guidelines [10]. If establishment of more standardized laboratory detection and clearer cut of values would reintroduce allelic ratio as guide for therapy in the areas of FLT3 inhibitors, remains to be answered by further clinical trials. At least data are supporting that FLT3 inhibitors seem particularly effective in patients with high *FLT3*-ITD allelic ratio [30,32].

3.3. Prognostic impact of *FLT3*-ITD insertion length

As the length of the ITD varies considerably among AML patients, the length of the ITD has been postulated to have a prognostic impact in AML. However, the impact of longer length of the *FLT3*-ITD is controversial, although have been associated with more autophosphorylation and thus poor survival outcomes [33]. The studies regarding the effect of ITD length have shown conflicting results, and the cutoff point to classify the ITD as «long» or «short» also lack standardization and clear consensus, although a commonly used cutoff point has been 48 base pairs (bps). In a recent systemic review regarding the length of the ITD, the authors concluded that patients with a long ITD have a moderate but statistically significantly higher risk of death, compared to patients with a short *FLT3*-ITD length [34]. ITD length is currently not included in standard prognostic scoring systems for AML [10], and it is not considered mandatory for laboratories to evaluate this in diagnostic work up for *FLT3* mutated AML [10]. However, further studies should consider this as a potential measurement for prognosis in AML.

3.4. Prognostic impact of the site of the *FLT3*-ITD

Most of the ITDs occur within the juxta membrane domain, where ITDs outside the JMDs occur in approximately 30% of cases. Studies have demonstrated that ITDs outside the JMDs, which include the TKDs, have been associated with resistance to chemotherapy and inferior outcome [35,36]. A recent studied conformed the negative impact of ITD involving the TKD, and furthermore, the negative prognostic impact of ITDs involving TKDs was not significantly affected by treatment with the FLT3 inhibitor midostaurin, as beneficial effect of midostaurin was only found for patients with JMD ITDs [37]. Hence, the site of the ITDs probably have prognostic impact; however, it is still unknown if ITDs involving TKDs are also associated with a lack of clinical effect of other FLT3 inhibitors and should be explored in further clinical trials [37].

3.5. Prognostic impact of concomitant *NPM1* mutation

The most common genetic mutation co-occurring with *FLT3* mutation is the *NPM1* mutation, which is found mutated in approximately 50–60% of *FLT3* mutated AML cases [7,8,24,25]. Also, in contrast to *FLT3* mutated AML, *NPM1* mutated AML is defined as an own disease entity in AML [10]. Studies of prognostic impact of coexistent *NPM1* mutation in *FLT3* mutated cases have been of conflicting results. Some studies have demonstrated no prognostic impact of *NPM1* mutation [29], while others have detected a benefit for *NPM1* mutation [38]. Based on a possible favorable prognostic impact of *NPM1* mutation in *FLT3* mutated cases, the ELN 2017 guidelines introduced a closed intricate classification system based on *FLT3* mutations and *NPM1* mutational status [9] (Table 2). However, in the recent guidelines, this is quite simplified, currently classifying *NPM1* mutated patients without *FLT3* mutations as favorable and *FLT3* mutated cases as intermediate regardless of the *NPM1* mutations [10] (Table 2). This can seem to be too simplistic and that some of the patients should be classified with more favorable prognosis and hence could be avoided to be refereed to an allo-HSCT in first remission. However, it should be emphasized that the evolving role of measurable residual disease (MRD) in AML therapy [39], and *NPM1* measurement by quantitative polymerase chain reaction (qPCR) is currently recommended in the follow up of AML patients with *NPM1* mutations [10]. The exact best timing, the cutoff values for detection, and the best interpretation of the MRD results are however still of debate [40,41]. The characteristic four base pair insertion in *NMP1* mutated AML allows mutation-specific assays to detect the mutant allele with little or no wild-type amplification, permitting sensitive assay. ELN 2022 guidelines recommend that MRD monitoring of *NPM1* by qPCR after two cycles of induction chemotherapy should be performed [10], and based on absence or presence of *NPM1* measurable transcripts, new evaluation should be performed. Relapse rates are high if *NPM1* mutant allele transcripts are detected [39], and many hematologist would recommend allo-HSCT for these patients if the patient is fit and a suitable donor is found [10,40,41]. Hence, MRD monitoring allows reprognostication of the patients based on initial chemotherapy response and guides the decision of the treating physicians regarding the choice of consolidation therapy. It should also be emphasized that relapse with other clones and other mutational profiles, not detected by PCR based MRD, are also described [40]. Finally, *FLT3* itself could be used to monitor treatment responses because it has been considered unlikely and of little value as markers of MRD, as the mutation probably almost always occurs relatively late in leukemogenesis, and because of the apparent instability. However, recent studies have brought new light and insight to this subject and have shown the potential possibility [42,43].

3.6. Prognostic impact of other genetic mutations

Other genetic alterations are also associated with *FLT3* mutations, and the most common seem to be *DNMT3A* occurring in about 30–40% of cases and *WT1* occurring in approximately 20% of cases. *TET2*, *NRAS*, *RUNX1*, *PTPN11*, and *ASXL1* are

occurring in approximately 10% of cases [4,24]. The prognostic impact of these other mutations in *FLT3 mutated AML* could be hard to predict, as the subgroups of patients are small and concurrence mutations across the mutational landscape exist [44]. However, for the occurrence of *WT1* mutations in *FLT3*-mutated cases, there seems to be a clear trend that this mutation is associated with a worse prognostic outcome [24]. The same is probably true for the *ASXL1* and *RUNX1* mutations, which are themselves associated with a poorer prognosis [10].

3.7. Prognostic impact of *FLT3* mutation among different cytogenetic subgroups

The role of the prognostic impact of different cytogenetics alterations has been well known for decades, and approximately 55–60% of AML patients have cytogenetic alterations at the time of diagnosis [45]. It should be emphasized that *FLT3* mutated AML patients could only be categorized as intermediate risk group in the absences of co-occurring adverse risk genetic lesions, including high risk cytogenetic alterations [10]. For cytogenetic aberrations associated with a favorable prognosis including t(8;21), inv16/t(16;16), and t(15;17), the *FLT3* mutations do not seem to have prognostic impact, which negatively affects the favorable prognosis associated with these genotypes leukemia [46]. A special high incidence of *FLT3*-ITD mutations is reported in AML patient with the t(6;9) aberration [47,48]. This translocation is associated with a poor prognosis and resistance to chemotherapy, and suitable patients should be offered allo-HSCT if possible [10,48]. Other cytogenetic alterations also appear in co-occurrence with *FLT3* mutations, and trisomy 8occurs in approximately 10% of cases, although it probably has less prognostic influence [49]. For cytogenetic features, associated with poor prognosis, like del5, del7, and complex karyotype, *FLT3* mutations do not impact the adverse outcome associated with these alterations [46]. Furthermore, the presence of high-risk cytogenetic aberrations might override *FLT3* mutations and lead to poorer response to conventional and targeted FLT3 therapy and hence inferior prognosis after allo-HSCT [50]. Whether FLT3 inhibitors have potential benefit in these patients and should be used in these settings, however, remains unanswered.

Table 3. Classification of FLT3 Inhibitors as first and second generation and type 1 and Type 2 inhibitors.

	Type 1	Type 2
First generation	Midostaurin (PKC412) Sunitinib Lestaurtinib	Sorafenib
Second generation	Gilteritinib Crenolanib	Quizartinib

4. FLT3 inhibitors

Since the discovery of the mutation the development of specific inhibitors of FLT3 has been considered as a desired therapeutic target for AML treatment, although just in recent years have these agentsentered clinical trials and altered clinical practice. Detailed descriptions of pharmacological properties of FLT3 inhibitors are beyond the scope of this article, and readers are directed to other comprehensive reviews [5,51]. In short FLT3 inhibitors can be divided into first and second generation, where the first are more broad-spectrum, multi-kinase inhibitors and the latter are more specific and potent and have fewer toxicities associated with off-target effects. Furthermore, they can be divided in type I and type II inhibitors, in which type I inhibitors bind to the ATP-binding site when the receptor is active, while type II inhibitors interact with a hydrophobic region immediately adjacent to the ATP-binding site that is only accessible when the receptor is in the inactive conformation. Table 3 demonstrates the classification of the most important FLT3 inhibitors in clinical use and clinical trials. These inhibitors have altered the treating paradigm for *FLT3* mutated AML significantly, and they have proven benefit in serval setting of *FLT3* mutated AML from induction therapy to post allo-HSCT maintenance therapy [6,25,52–55]. Table 4 summarizes different clinical settings of FLT3 therapy in AML and key clinical studies. Based on clinical trials so far, midostaurin has generally been recommended in induction regimen, gilteritinib as salvage therapy for relapsed and refractory cases, and sorafenib or midostaurin in maintenance therapy after allo-HSCT [10,56]. However, it should be noticed that primary resistance or development of resistance to FLT3 inhibitors are of considerable clinical concerns [57,58]. Future strategies to overcome and bypass these resistance mechanisms are greatly needed, and translational and clinical research is therefore of great importance in this setting [57,59]. Finally, effect of FLT3 inhibitors is also affected by other cytogenetical alterations and genetical mutation [50]

Table 4. FLT3 inhibitors used in different clinical settings of FLT3 therapy in AML and key clinical studies.

Setting	Inhibitors	Comments	Key references:
Induction	Midostaurin	Addition midostaurin to standard chemotherapy significantly prolonged overall and event-free survival among *FLT3* mutated patients.	[6]
Unfit	Gilteritinib	Gilteritinib + azacitidine resulted in significantly higher composite complete remission rates, compared to azacitidine alone in *FLT3*-mutated patients unfit for intensive chemotherapy.	[52]
Relapsed/ refractory	Gilteritinib	Gilteritinib resulted in significantly longer survival and higher remission rates compared to salvage chemotherapy alone for relapsed or refractory *FLT3*-mutated patients.	[53]
Post allo-HSCT	Sorafenib	Sorafenib maintenance post-transplantation significantly reduced relapse in patients with *FLT3*-ITD AML undergoing allo-HSCT.	[54]
	Midostaurin	Potential increased relapsed free survival with midostaurin in patients with *FLT3*-ITD AML undergoing allo-HSCT.	[55]

and which subgroups are most suitable for FLT3 therapy remains to be established in clinical trials.

5. Expert opinion

The possible prognostic significance of *FLT3* mutation in AML has been under debate and up for revision several times since the discovery of its existence in AML. Initial studies showed that the patient with *FLT3*-ITD had a poor outcome from treatment and was often associated with refractory and relapsing disease. This is also reflected in the ELN's previous consensus program for AML [8]. Gradually, studies emerged demonstrating that the picture was somewhat more complex and heterogeneous [9]. Aspects related to *FLT3-ITD* length, location, and allelic ratio, in addition to coexistence of other genetic alterations have all been investigated. At the same time, new approaches have emerged and altered the landscape of *FLT3* mutated AML, especially the introduction of FLT3 inhibitors and establishment of MRD monitoring. In the recent 2022 ELN guidelines, this has been taken into account [10,60] and accordingly the last decade has seen improved treatment results for *FLT3* mutated AML patients. The recommendation relating to the prognostic significance of both the *FLT3* mutation allele ratio and impact of coexistence *NPM1* mutation was removed from the updated guidelines. Although this brings hopes that the future will further improve the treatment outcome for patients with *FLT3* mutated AML, some aspects are still to be answered. One of the main questions is still remaims, that is, which patients should be offered allo-HSCT in first remission? Although the results for allo-HSCT are still improving, the treatment is still associated with a high degree of treatment-related morbidity and mortality. Whether we in the future could treat these patients with satisfactory results without offering allo-HSCT, with the accompanying risk it entails, remains an unanswered question [11]. Future research is strongly needed to continue to offer these AML patients the best available treatment options.

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ORCID

Håkon Reikvam http://orcid.org/0000-0001-5439-8411

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