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REVIEW ARTICLE

Dynamics of *FLT3* mutations in acute myeloid leukemia: A systematic review and meta-analysis of shifts between diagnosis and relapsed/refractory disease

Lais Teixeira, Camilla Correia, Alini Ponte, Diego Miranda, Felipe Feistauer, and Marco Aurélio Salvino*

Postgraduate Program in Medicine and Health, Professor Edgard Santos University Hospital, Medical School, Universidade Federal da Bahia (UFBA), Salvador (BA), Brazil

Abstract

Acute myeloid leukemia (AML) is a hematologic malignancy with a generally poor prognosis. Technological advances in molecular diagnosis have identified genetic alterations driving AML pathogenesis, among which FMS-like tyrosine kinase 3 (*FLT3*) mutations are significant. These mutations hold prognostic value and are increasingly recognized as potential markers for disease monitoring. This systematic review and meta-analysis aimed to assess the prevalence of changes in *FLT3* mutational status in adult patients with relapsed or refractory AML compared to their status at initial diagnosis. A relevant proportion of patients who were *FLT3*-wildtype at diagnosis were found to be *FLT3*-mutated on relapse, emphasizing the importance of continuous mutation monitoring. Subgroup analyses were also performed, and mutation shift rates were reported across both *FLT3* internal tandem duplication and tyrosine kinase domain subtypes. These findings illustrate the genetic evolution of leukemic clones and support the need for tailored therapeutic approaches based on the mutational profile at different disease stages. This study further highlights the diagnostic and clinical utility of routine molecular reassessment and offers practical recommendations for integrating *FLT3* retesting into standard AML management.

Keywords: Acute myeloid leukemia; FMS-like tyrosine kinase 3 mutation; FMS-like tyrosine kinase 3; Molecular biology

***Corresponding author:**
Marco Aurélio Salvino
(marcohemato@ufba.br)

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1. Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy that primarily affects elderly individuals over the age of 60 years. Despite advances in supportive care therapies, AML remains associated with a poor prognosis, with an estimated 5-year overall survival rate of approximately 40%.[1] Studies conducted at the University Hospital of the Federal University of Bahia reported a median overall survival of only 7–8 months among adult patients.[2,3] Given this clinical reality, a deeper understanding of the disease's pathophysiology is essential for identifying prognostic markers and therapeutic targets.

Advancements in molecular biology have significantly reshaped the AML landscape, particularly by enabling the identification of actionable mutations that may be targeted

to improve patient survival. The success of targeted therapy in chronic myeloid leukemia, for example, where the discovery of the *BCR/ABL* fusion gene led to the development of tyrosine kinase inhibitors (TKIs), illustrates the transformative potential of molecularly guided treatments.[4]

FMS-like tyrosine kinase 3 (*FLT3*) mutations, first described in 1996,[5] are now recognized as the most common genetic alterations in AML. These mutations are associated with adverse prognostic implications and poor overall survival.[6] FLT3 belongs to the class III receptor tyrosine kinase family. These receptors are characterized by a membrane-bound structure comprising five extracellular immunoglobulin-like domains and an intracellular catalytic region. This catalytic region is divided into two kinase domains (TKD1 and TKD2), separated by a hydrophilic insert of variable length. Members of this receptor family include FMS (macrophage colony-stimulating factor receptor), platelet-derived growth factor receptor α and β, KIT (stem cell factor receptor), and FLK-2/FLT3.[7,8] These proteins play a pivotal role in the regulation of hematopoietic cell proliferation, differentiation, and survival.

Structurally, FLT3 comprises an extracellular transmembrane domain, a juxtamembrane domain (JMD) within the cytoplasm, and two intracellular tyrosine kinase domains (TKD1 and TKD2), which are connected through a linker segment. The JMD is further subdivided into three functional regions: A binding site (JM-B), involved in receptor activation and stabilization of the inactive conformation; a switching region (JM-S), which includes phosphorylation sites and a binding site for STAT5; and a ligand segment (JM-Z). Mutant FLT3 receptors exhibit constitutive, ligand-independent activation that contributes to leukemogenesis by promoting uncontrolled proliferation (class I mutations). In contrast, class II mutations impair hematopoietic differentiation. Importantly, class I mutations typically occur at later stages of leukemogenesis and require coexisting molecular lesions to fully transform hematopoietic progenitors into leukemic clones. Two main classes of mutations have been identified. The first comprises internal tandem duplications (ITDs), most commonly affecting exons 14 and 15 within the JMD. *FLT3*-ITD mutations are observed in approximately 25% of AML cases. The second class involves point mutations within the activation loop of the TKD, particularly in TKD2, and occurs in about 5% of cases. These are collectively referred to as *FLT3*-TKD mutations.[9,10]

FLT3 mutations–particularly the ITD subtype–have been consistently associated with leukocytosis at diagnosis. Although induction therapy often results in similar initial complete remission rates (~70%) regardless of *FLT3* mutational status, patients harboring *FLT3*-ITD mutations exhibit significantly higher relapse rates. One study reported a median disease-free survival of 8.4 months in *FLT3*-mutated AML versus 12.8 months in wild-type cases. Similarly, overall survival was reduced in *FLT3*-mutated patients (12 months vs. 15 months).[11-13] *FLT3*-ITD mutations are more frequently observed in *de novo* AML compared to AML secondary to myelodysplasia. *FLT3*-TKD mutations, on the other hand, have demonstrated less consistent prognostic impact. The limited number of patients with these mutations in published studies hinders the ability to draw robust conclusions. Unlike *FLT3*-ITD, *FLT3*-TKD is not associated with elevated leukocyte counts, possibly reflecting distinct downstream signaling mechanisms.[14]

The therapeutic landscape of AML with *FLT3* mutations has evolved significantly with the advent of targeted agents, resulting in substantial improvements in patient outcomes. Phase III clinical trials in both newly diagnosed and relapsed/refractory settings have demonstrated that FLT3 inhibitors increase the likelihood of achieving measurable residual disease (MRD)-negative remission, even in the absence of allogeneic hematopoietic stem cell transplantation.

In the relapse setting–historically associated with extremely poor outcomes–the availability of FLT3 inhibitors has translated into improved remission rates.[15] and survival, particularly in patients ineligible for transplantation. Moreover, responses to these agents have been associated with enhanced performance status and quality of life, allowing some patients previously deemed unfit to become eligible for curative-intent transplant procedures. This is particularly relevant, given that allogeneic stem cell transplantation remains the only potentially curative strategy in relapsed AML. Despite these advances, the long-term prognosis in this patient population remains poor, with overall cure rates below 10%.[16] Ongoing studies are investigating various combination regimens involving FLT3 inhibitors, as monotherapy–though approved in many countries, including Brazil–is generally insufficient for achieving durable remissions in most cases.[17]

Evidence suggests that *FLT3* mutational profiles may change over time, with patients either losing or acquiring *FLT3* mutations at relapse despite their initial diagnostic status.[18] These changes have important therapeutic implications. Several targeted agents–such as midostaurin, gilteritinib, and quizartinib–have been developed for patients with *FLT3* mutations. As a result, understanding the dynamics of *FLT3* mutational status and incorporating

routine reassessment using polymerase chain reaction (PCR) and next-generation sequencing (NGS) may inform prognosis and guide therapeutic strategies. In some cases, such reassessment may enable the use of FLT3 inhibitors, which can enable the patient to undergo curative treatment - bone marrow transplant.

Beyond their prognostic therapeutic value, *FLT3* mutations have also been investigated as potential markers for disease monitoring. Currently, treatment response is often assessed through flow cytometry-based immunophenotyping to detect MRD through abnormal surface markers. This approach has been validated as a prognostic tool that correlates with relapse risk and treatment response.[18] The combination of flow cytometry with molecular assays—such as PCR and NGS—further enhances prognostic accuracy. Some mutations, such as those in the *NPM1* gene, are considered reliable MRD markers.[19] However, the role of *FLT3* mutations in this context remains controversial due to their unstable behavior during disease evolution.

Reassessment of *FLT3* mutational status at relapse is critical, given its profound therapeutic implications. The clinical course of patients with *FLT3*-mutated relapse who do not receive a FLT3 inhibitor is uniformly poor, and targeted therapy may represent the only viable option for disease control or remission. Importantly, evidence suggests that *FLT3* mutations may emerge at relapse in patients who were *FLT3*-wild type at diagnosis, highlighting the dynamic nature of the leukemic clone. Although current data remain limited and somewhat inconclusive, they underscore the necessity of routine molecular reassessment during disease progression. These dynamic shifts in clonal architecture have profound clinical implications. The acquisition of new mutations—either within *FLT3* or in other oncogenic drivers—can lead to increased proliferation, therapeutic resistance, and disease aggressiveness. For example, secondary *FLT3*-TKD mutations (e.g., N676K, F691L, A627P, Y842C) may arise during treatment with midostaurin or second-generation FLT3 inhibitors, conferring resistance.[20-22] In parallel, mutations in other genes, such as *ASXL1*, *WT1*, and *KMT2A*, may further impair prognosis and treatment responsiveness.[23]

Despite current recommendations advocating for comprehensive molecular profiling in all cases of newly diagnosed AML—particularly in patients with cytogenetically normal AML (CN-AML)—the universal implementation of *FLT3* testing remains suboptimal. Retrospective registry data have indicated that in some centers, only 77% of patients with CN-AML underwent *FLT3* mutational analysis. Alarmingly, this disparity is more pronounced when comparing academic centers to community-based institutions, likely reflecting unequal access to molecular diagnostics and variable awareness regarding the clinical utility of these tests.[24,25]

Additional data from a cohort at the Hospital das Clínicas of the Federal University of Bahia (UFBA) revealed that nearly half (47%) of patients could not be adequately risk-stratified according to the 2022 European Leukemia Net guidelines due to the absence of molecular data. Furthermore, only 21% of patients had any molecular reassessment throughout their disease.[2] Although the recommendation to perform *FLT3* testing in all CN-AML cases dates back to 2010, this gap in implementation underscores a persistent challenge in real-world practice. These shifts in the mutational profile underscore the need for routine molecular reassessment, especially in the setting of relapse or refractory disease.

Given the increasing availability of *FLT3*-targeted therapies, identifying the mutational status at each disease stage is critical. Previous studies suggest that *FLT3* mutations may emerge at relapse in initially *FLT3*-wild type patients. Timely identification of an actionable *FLT3* mutation may significantly impact therapeutic decision-making and alter the clinical course—even offering curative options in otherwise adverse scenarios. Understanding this shift is critical for tailoring therapy.

This study aims to systematically evaluate published evidence on the dynamics of *FLT3* mutations in adult patients with AML, comparing the mutation status at diagnosis and relapse or refractory disease. The objectives are to (i) quantify the *FLT3* mutation shift rate, (ii) distinguish changes between *FLT3*-ITD and *FLT3*-TKD subtypes, (iii) assess methodological variability, (iv) explore corresponding clinical implications, and (v) provide evidence-based recommendations for routine molecular retesting.

2. Methods

2.1. Search strategy

An initial literature search was conducted in June 2020 using the PubMed database to identify studies relevant to the research area. Search terms were selected based on Medical Subject Headings (MeSH) and included: "fms-like tyrosine kinase 3" OR "Fetal Liver Kinase-2" OR "Fetal Liver Kinase 2" OR "Fetal Liver Kinase-3" OR "Fetal Liver Kinase 3" OR "CD135 Antigens" OR "Antigens, CD135" OR "CD135 Antigen" OR "Antigen, CD135" OR "Stem Cell Tyrosine Kinase 1" OR "FLT3" AND "Leukemia, Myeloid, Acute"[MeSH] OR "Acute Myeloid Leukemia" OR "ANLL" OR "Leukemia, Acute Myelogenous" OR

"Leukemia*, Acute Myeloid" OR "Leukemia, Myeloblastic, Acute" OR "Leukemia, Myelocytic, Acute" OR "Leukemia, Myelogenous, Acute" OR "Leukemia, Nonlymphoblastic, Acute" OR "Leukemia, Nonlymphocytic, Acute" OR "Myeloblastic Leukemia*, Acute" OR "Acute Myeloblastic Leukemia*" OR "Leukemia*, Acute Myeloblastic" OR "Myelocytic Leukemia*, Acute" OR "Acute Myelocytic Leukemia*" OR "Leukemia*, Acute Myelocytic" OR "Myelogenous Leukemia, Acute" OR "Myeloid Leukemia*, Acute" OR "Nonlymphoblastic Leukemia*, Acute" OR "Acute Nonlymphoblastic Leukemia*" OR "Leukemia*, Acute Nonlymphoblastic" OR "Nonlymphocytic Leukemia*, Acute" OR "Acute Nonlymphocytic Leukemia*" OR "Leukemia*, Acute Nonlymphocytic" OR "Acute Myelogenous Leukemia*" OR "Leukemias, Acute Myelogenous" OR "Myelogenous Leukemias, Acute" OR "Myeloid Leukemia, Acute, M1" OR "Leukemia, Myeloid, Acute, M1" OR "Acute Myeloid Leukemia without Maturation" OR "Leukemia, Myeloid, Acute, M2" OR "Myeloid Leukemia, Acute, M2" OR "Acute Myeloid Leukemia with Maturation*" AND "Recurrence"[MeSH terms] OR "Recrudescence" OR "Recrudescences" OR "Recurrences" OR "Relapse" OR "Relapses."

No date restriction was applied. An updated search was conducted in July 2024 to include newly published articles. After the initial screening of titles and abstracts, eligible articles were selected for full-text review. No articles were excluded due to sample size. No particular subgroup of AML was selected for analysis.

2.2. Inclusion and exclusion criteria

Studies were included if they: (i) investigated adult patients with AML; (ii) were written in English, Spanish, or Portuguese; (iii) employed PCR as the method for detecting *FLT3* mutations, as it is considered the current gold standard; and (iv) reported *FLT3* mutational status at both diagnosis and relapse/refractory stages. The exclusion criteria were: (i) studies exclusively involving pediatric populations, (ii) studies focusing solely on acute promyelocytic leukemia; (iii) phase I interventional clinical trials; (iv) preclinical studies; (v) review articles, letters to the editor, and case reports; and (vi) studies lacking data on paired samples or failing to report *FLT3* mutational status at both relevant time points.

2.3. Study selection and data extraction

Articles were screened by one of the authors (L.T.). The selection process involved reviewing the title and abstract, followed by a methodological analysis of each study to identify the technique used for mutation analysis (PCR vs. NGS) and whether the evaluation was performed using paired samples. After the initial selection, full-text articles were read to ensure eligibility. Subsequently, the reference lists of each included article were reviewed to identify any additional studies that may have been missed during the database search.

Data were collected from retrospective, prospective, or interventional studies involving patients with relapsed/refractory AML. Extracted data included study design, year of publication, number of patients, *FLT3* mutational status at diagnosis and relapse using paired samples, type of *FLT3* mutation (ITD, TKD, or wild-type), as well as karyotype, French–American–British (FAB) classification, and allelic burden. *FLT3* mutational status was considered to have changed if paired samples from the same patient displayed different results at diagnosis and the relapse/refractory stage. The study's article inclusion flowchart, based on PRISMA methodology, is presented in Figure 1.

3. Results

Following the initial search conducted in 2020, 1,201 articles were identified. After screening the titles and abstracts, 513 were selected for further analysis. Of these, after evaluation of the methodology and results, 12 articles were eligible for full-text review, and an additional five were retrieved through manual reference list searches. Among the full-texts reviewed, five were excluded due to a lack of relevant data on *FLT3* mutational status, and one due to divergent population selection criteria.

In the updated 2024 search, 1,641 articles were retrieved. After screening, 161 were selected based on titles and abstracts. Of these, 56 underwent full-text analysis; 19 studies were excluded for not using PCR as the detection method, 10 overlapped with the initial search, and 16 did not report paired sample data or detailed mutational profiles. In addition, two were excluded for comparing different PCR techniques, and nine were ultimately included. In total, 20 studies met the inclusion criteria (Figure 1).

All included studies employed PCR as the method for *FLT3* mutational analysis; 11 studies assessed both *FLT3*-ITD and *FLT3*-TKD, eight assessed only *FLT3*-ITD, and one focused exclusively on *FLT3*-TKD (Table 1). The year of publication of the works selected in this study ranged from 1999 to 2014. Only three studies were prospective; the others were retrospective. Just one study that evaluated both *FLT3*-ITD and *FLT3*-TKD indicated robust longitudinal data with paired samples.

The quality of the studies varied substantially. Only one-third of publications had samples larger than 100 patients. However, several studies with smaller numbers of patients (<50) were included for data aggregation. The study by

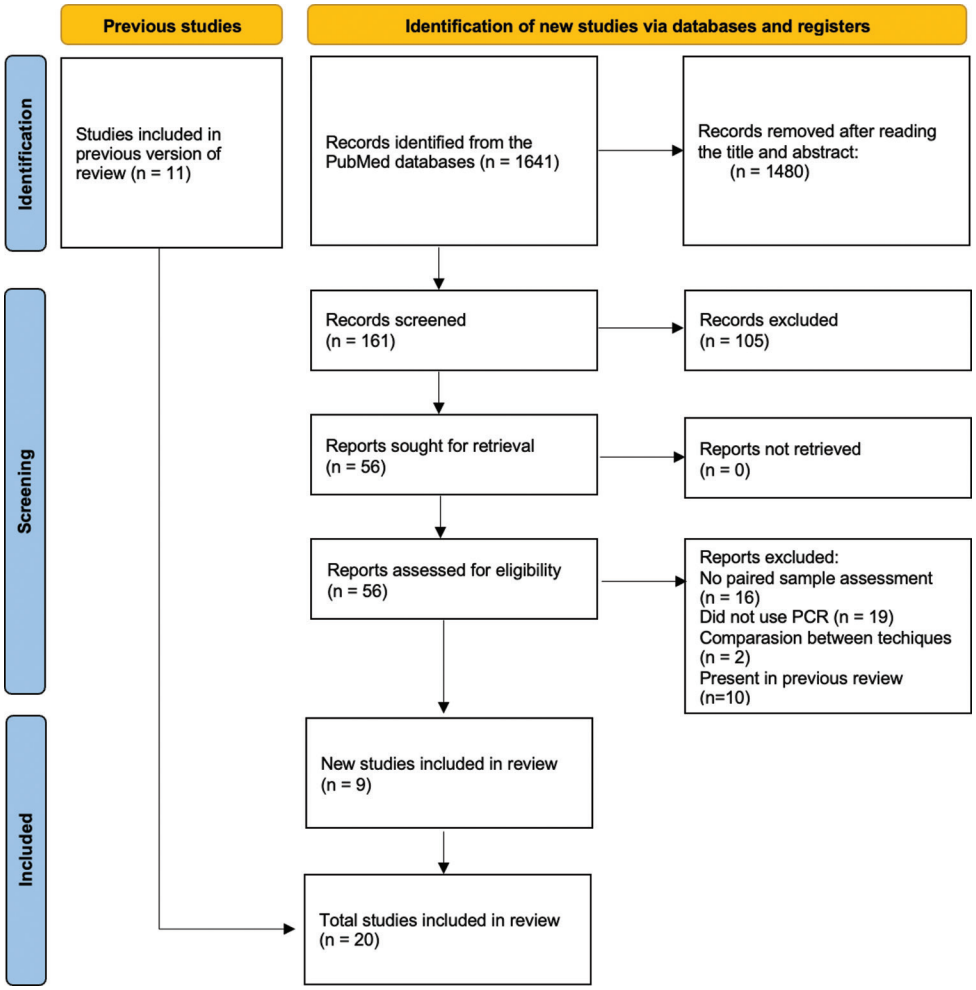

Figure 1. Flowchart of study selection and inclusion based on eligibility criteria for systematic review
Abbreviation: PCR: Polymerase chain reaction.

Shih *et al.*[45] focused on data from *FLT3*-TKD and included a robust patient cohort, providing valuable data to the analysis—particularly important given that TKD was the least studied *FLT3* mutation among the selected articles.

A total of 1,094 patients with relapsed or refractory AML were included. At diagnosis, 294 patients (26%) were *FLT3*-ITD-positive and 30 (3%) were *FLT3*-TKD-positive. At first relapse or refractory disease, 317 (29%) were *FLT3*-ITD-positive and 31 (3%) were *FLT3*-TKD-positive, in agreement with data described in the literature. A change in mutational status was observed in 147 patients (13%), indicating that approximately one in seven patients experienced a shift in *FLT3* status. Notably, 5% of patients had missing mutational data at relapse. Pediatric patients ($n = 42$) included in the study by Cloos *et al.*[30] were excluded from the final analysis.

Among patients for whom *FLT3*-ITD evolution could be evaluated ($n = 936$), 222 (24%) maintained the mutation, 46 (5%) became negative, and 73 (8%) acquired the mutation during disease progression, indicating a 13% mutation shift rate (*i.e.*, one in seven patients) (Figure 2).

In the subset of 543 patients with *FLT3*-TKD data (reported in only 10 studies), 14 (2%) retained the mutation, 20 (3%) lost it, and 15 (3%) acquired it at relapse—a shift rate of 6%, or approximately one in 17 patients (Figure 3).

In addition, five cases of double mutation (*FLT3*-ITD/TKD) exhibited dynamic changes; three patients were *FLT3*-ITD/TKD-positive at diagnosis and became *FLT3*-ITD-positive with *FLT*-TKD negative; one patient acquired the double mutation at relapse, and another one who at relapse became negative for both mutations.

Overall, 36% of the studied population tested positive for the *FLT3* mutation at some point during the disease course.

For a more accurate analysis, we extracted individual patient data, some of which included information on

Table 1. Summary of included studies

Study	Year of publication	Number of patients	Mutation (s) evaluated	References
Kottaridis *et al.*	2002	44	*FLT3*-ITD; *FLT3*-TKD	26
Nakamura *et al.*	2004	24	*FLT3*-ITD; *FLT3*-TKD	27
Tiesmeier *et al.*	2004	31	*FLT3*-ITD; *FLT3*-TKD	28
Suzuki *et al.*	2005	39	*FLT3*-ITD; *FLT3*-TKD	29
Cloos *et al.*	2006	38	*FLT3*-ITD; *FLT3*-TKD	30
Palmisano *et al.*	2007	28	*FLT3*-ITD; *FLT3*-TKD	31
Schnittger *et al.*	2009	80	*FLT3*-ITD; *FLT3*-TKD	32
McCormick *et al.*	2010	50	*FLT3*-ITD; *FLT3*-TKD	33
Wang *et al.*	2010	17	*FLT3*-ITD; *FLT3*-TKD	34
Wakita *et al.*	2012	34	*FLT3*-ITD; *FLT3*-TKD	35
Janke *et al.*	2014	156	*FLT3*-ITD; *FLT3*-TKD	36
Nakano *et al.*	1999	28	*FLT3*-ITD	37
Shih *et al.*	2002	108	*FLT3*-ITD	38
Schnittger *et al.*	2004	97	*FLT3*-ITD	39
Park *et al.*	2011	69	*FLT3*-ITD	40
Abdelhamid *et al.*	2012	8	*FLT3*-ITD	41
Bachas *et al.*	2012	6	*FLT3*-ITD	42
Nazha *et al.*	2012	102	*FLT3*-ITD	43
Gourdin *et al.*	2014	15	*FLT3*-ITD	44
Shih *et al.*	2004	120	*FLT3*-TKD	45

karyotype and FAB classification profile. From this dataset, we had a total of 384 patients. Among patients with *FLT3*-ITD at diagnosis ($n = 160$), 123 had their FAB classification described, with types M2, M1, and M4 being the most frequent, at 29%, 27%, and 22%, respectively. Among ITD patients who switched to TKD, one was M5 and the other M4. Among ITD cases that became negative, five were M4 and eight were M1. Among TKD cases that converted to ITD, two were M2, one was M5, and one was M4. Among TKD patients at diagnosis who became negative, four were M1. There were a total of five patients with double mutation, all M2 at diagnosis, four with normal karyotype, and one with *MLL*-PTD mutation.

When analyzing the karyotype of these patients, among the nine with available data who changed from ITD to negative, two had the +8 mutation, which persisted at relapse, two displayed normal karyotype at both diagnosis and relapse, and one changed from normal karyotype to complex. Among patients who were negative at diagnosis and acquired the ITD mutation at relapse, four had normal karyotypes, none had a complex karyotype, and one had core binding factor t(8;21), which remained after relapse. Out of a total of 26 patients, 14 had missing karyotype data at some point in the disease. Among those who converted to TKD ($n = 10$), two had a karyotype that changed from normal at diagnosis to complex at relapse, and one maintained a complex karyotype throughout the disease. The study by Nakano *et al.*,[37] despite a small number of patients, was one of the first to perform a paired assessment of molecular alterations in AML; however, it used cytogenetic alterations in the progression of the disease as the primary analysis.

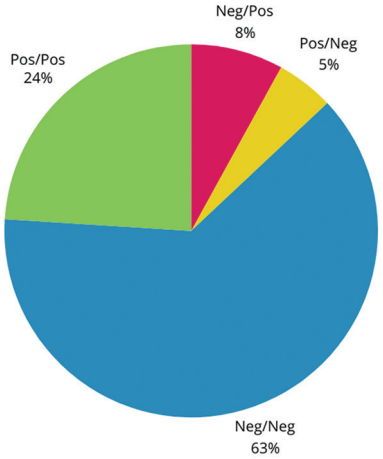

Figure 2. Change in *FLT3*-ITD mutational status between diagnosis and relapse/refractory disease

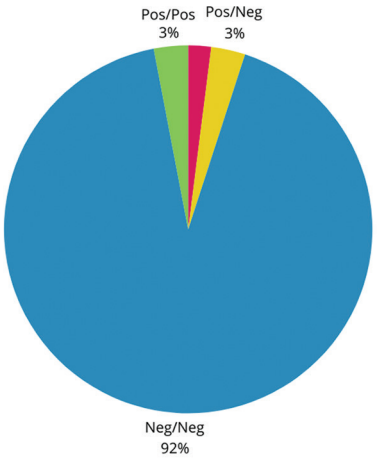

Figure 3. Change in *FLT3*-TKD mutational status between diagnosis and relapse/refractory disease

All studies were cohort designs and evaluated using the Newcastle-Ottawa Quality Assessment Scale (Table 2).

4. Discussion

The present study is the first systematic review and meta-analysis investigating *FLT3* mutational shifts in AML, incorporating data from over 1,000 patients. The prevalence of *FLT3* mutations observed aligned with previous reported rates in the literature. Notably, our analysis revealed that approximately one in seven patients exhibited a change in *FLT3* mutation status between diagnosis and relapse or refractory disease. Such a frequency is not negligible and reinforces the recommendation for molecular reassessment at multiple time points during the disease course. The increasing use of mutation-guided therapies in AML underscores the need for accurate and dynamic mutational profiling. Identifying the emergence or loss of actionable mutations can prevent the administration of potentially toxic therapies with limited benefit in mutation-negative patients, and conversely, guide the appropriate use of targeted agents in those who acquire relevant mutations.

Table 2. Newcastle–Ottawa Scale quality assessment scores for included studies

Study	Selection	Comparability	Outcome	References
Shih *et al.*	****	*	***	45
Nazha *et al.*	****	*	**	43
Shih *et al.*	****	*	***	38
Nakano *et al.*	****	*	***	37
McCormick *et al.*	****	*	***	33
Cloos *et al.*	****	*	***	30
Palmisano *et al.*	****	*	***	31
Suzuki *et al.*	****	*	***	32
Tiesmeier *et al.*	****	*	***	28
Kottaridis *et al.*	****	*	***	26
Schnittger *et al.*	**	*	***	32
Gourdin *et al.*	****	*	***	44
Janke *et al.*	****	*	**	36
Wakita *et al.*	****	*	***	35
Park *et al.*	****	**	***	40
Wang *et al.*	***	**	***	34
Schnittger *et al.*	****	**	***	32
Bachas *et al.*	***	*	***	42
Abdelhamid *et al.*	***	*	***	41
Nakamura *et al.*	*	*	**	27

Notes: The Newcastle–Ottawa Quality Assessment Scale checklists adapted for cross-sectional and cohort studies. *Asterisks correspond to ratings assigned for each item according to The Newcastle–Ottawa Quality Assessment Scale.

These findings have therapeutic implications and can impact patient survival. In some cases, identifying a newly acquired mutation could provide eligibility for the only potentially curative option in relapsed or refractory AML (i.e., bone marrow transplantation).

This discrepancy may be associated with inherent limitations of the detection method itself. It is well known that molecular technologies have improved over the years. Currently, the most recommended PCR method for *FLT3* analysis is fragment analysis. In our evaluation, some older studies used gel-based PCR as the detection method, which has lower sensitivity. It is also important to consider the possibility of false negatives. PCR is a method that has difficulty detecting very short fragments, identifying mutations in previously undescribed regions, and provides only limited information, such as size, number, and allelic burden. It does not provide additional details about the sequence or its specific location, nor can it distinguish between homozygous and heterozygous mutations. Nevertheless, PCR is a more accessible method compared to NGS and yields results more rapidly. Therefore, the sensitivity of the technique may influence the findings.

Another contributing factor is the nature of the mutation itself. Some studies suggest that *FLT3* mutations may emerge later in the course of AML, following a branched clonal evolution model. This may occur due to external factors, such as chemotherapy or alterations in the bone marrow microenvironment. For example, a new subclone or one that was present at undetectable levels may acquire a selective advantage over other clones, leading to its proliferation and potential dominance. Alternatively, the mutation may already be the predominant clone at diagnosis. In such cases, it may be eliminated by treatment or reduced to levels below the detection limit.

There is an ongoing debate in the literature regarding the utility of *FLT3* as a measurable residual disease marker and its prognostic significance in AML. The previously described instability in its mutational status limits its application for MRD monitoring.

Some studies have suggested that the acquisition of *FLT3* mutations in relapsed AML is associated with an adverse prognosis,[46] with outcomes comparable to patients who remain *FLT3*-mutated at both diagnosis and relapse. Notably, the absence of *FLT3* mutations at relapse has been associated with a more favorable prognosis when compared to patients who remain FLT3-mutated.[9] In the current review, it was not possible to evaluate clinical outcomes such as overall survival, as only a minority of studies reported this information. Furthermore, data regarding cytogenetic and cytomorphologic changes were insufficient for analysis, as these parameters were not mandatory

inclusion criteria in most studies. Consequently, only descriptive analysis was feasible. In addition, the impact of FLT3 allelic burden on prognosis could not be assessed due to a lack of extractable data.

In all studies included in the present review, *FLT3* mutation detection was performed using PCR, the most widely adopted method in clinical practice. With the advancement of molecular diagnostics, more sensitive techniques have emerged, offering enhanced mutation detection and broader genomic insights beyond the scope of conventional PCR. NGS, for example, has gained traction in AML diagnostics; however, its sensitivity in detecting *FLT3*-ITD mutations is limited by mutation size and allele frequency. Moreover, studies have highlighted variability even among PCR-based assays. The PCR method of choice for this type of assessment is fragment analysis PCR, which is more modern and sensitive. In the present review, some of the included studies were older and employed gel-based PCR, which may have contributed to false-negative results. In the context of clinical research, there has been a growing trend toward combining PCR and NGS methodologies, thereby improving mutation detection and enabling more refined genomic characterization. As previously reported,[47,48] this hybrid approach holds clinical relevance in identifying resistance profiles to TKIs and may become the new gold standard for *FLT3* mutation analysis.

FLT3 inhibitors have demonstrated promising results in clinical trials and are increasingly integrated into therapeutic protocols, with regulatory approvals in multiple regions. In Brazil, midostaurin is currently approved for newly diagnosed patients harboring *FLT3*-ITD or *FLT3*-TKD mutations, based on the RATIFY study, which demonstrated an improvement in overall survival and event-free survival compared to placebos. For relapsed/refractory AML, gilteritinib has been approved for patients with *FLT3* mutations, based on the ADMIRAL study, which reported an overall survival of 9.3 months compared to 5.6 months with salvage chemotherapy.[49]

Our findings demonstrated a mutational status change in 14% of patients, highlighting the importance of routine molecular reassessment at relapse. Despite its relevance, access to *FLT3* mutational status testing remains limited in many clinical settings. In the Brazilian public healthcare system, previous data have indicated that up to 79% of patients lack adequate molecular characterization due to infrastructural and technological constraints.[2] In the present review, approximately 5% of patients had incomplete molecular data. The disparity is even more pronounced when comparing academic versus non-academic centers—a global challenge in cancer care. A study conducted in the United States demonstrated a lower rate of testing for AML patients in academic centers compared to community centers. This discrepancy may reflect the heterogeneous incorporation of testing technologies across treatment facilities and a potentially lower rate of molecular testing requests by medical teams in community settings, as academic institutions often require more frequent medical updates.

In Brazil, the public healthcare system, known as the Unified Health System (SUS), faces significant challenges in the incorporation of new technologies, even within academic centers. For example, cytogenetic analysis—recommended since the 1980s—is still not fully implemented in the public system. This was evidenced in a study conducted at the academic center of the University Hospital of Bahia, where only 60% of patients underwent karyotype analysis at diagnosis.[2] While investment in technological infrastructure is essential, parallel efforts are required to enhance continuous medical education, ensuring that healthcare providers recognize the clinical utility of molecular data for risk stratification and therapeutic decision-making.

A key limitation of this systematic review is that the screening of titles and abstracts was performed by a single investigator, which may have introduced selection bias and potentially resulted in the exclusion of relevant studies. As is widely recognized, systematic reviews require rigorous methodology, ideally involving independent review by multiple researchers to minimize bias. Moreover, some studies were excluded due to missing or incomplete data. This review could not be registered on the PROSPERO platform, as it currently only accepts registrations of systematic reviews and meta-analyses involving interventions. The present work is classified as a systematic review and meta-analysis with aggregation of prevalence data, in which the total proportion of *FLT3*-mutated and *FLT3*-wildtype patients was calculated. As a descriptive prevalence meta-analysis, it is not possible to perform statistical analyses to assess heterogeneity or publication bias. This methodological choice was based on the clinical relevance of the data, which focused on mutation frequency. To strengthen the findings, future studies should involve more than one reviewer and incorporate complementary clinical outcome data to enable more robust statistical analyses.

5. Conclusion

The characterization of *FLT3* mutation status and subtype in AML represents one of the most robust molecular models for supporting diagnosis, refining disease pathophysiology, guiding risk stratification, and informing therapeutic

decision-making, including the indication for allogeneic hematopoietic stem cell transplantation. Additionally, *FLT3* mutational profiling plays a pivotal role in selecting targeted therapies that are either currently approved or under investigation.

In this context, the observation that one in seven patients exhibited a change in *FLT3* mutational status between diagnosis and relapse/refractoriness underscores the clinical relevance of routine molecular reassessment at disease progression. Such alterations may reflect the expansion of pre-existing subclones at diagnosis or the acquisition of *de novo* mutations associated with distinct leukemic biology. The prognostic implications of these molecular shifts remain to be fully elucidated and warrant further investigation.

There remains a pressing need to advance the sensitivity and breadth of diagnostic tools capable of detecting minor subclonal populations at disease onset, as well as to better characterize the mutational architecture and its dynamics over time. The incorporation of next-generation molecular technologies into healthcare systems is essential.

Equally important is the continuous education of healthcare professionals to ensure the appropriate interpretation and integration of these tools into clinical practice. Routine assessment of *FLT3* mutations throughout the disease course is critical for optimizing the monitoring and management of patients with AML.

We suggest further studies, including prospective cohort studies integrating PCR with NGS, along with more robust investigations incorporating cytogenetic analyses at both diagnosis and relapse, co-occurring molecular alterations, and refined risk stratification models.

Based on these findings, the prevalence of *FLT3* mutational profile changes is clinically relevant. We therefore recommend performing PCR with fragment analysis to assess *FLT3* mutations in all patients with AML, both at diagnosis and at relapse or refractory disease.

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Conflict of interest

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Author contributions

Conceptualization: Lais Teixeira, Camilla Correia, Marco Salvino
Formal analysis: Lais Teixeira
Methodology: Lais Teixeira
Writing–original draft: All authors
Writing–review & editing: Lais Teixeira, Marco Salvino

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