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ORIGINAL ARTICLE

Upfront intensive treatment analysis of the Italian Cohort Study on *FLT3*-mutated AML patients (FLAM): The impact of a FLT3 inhibitor addition to standard chemotherapy in the real-life setting

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Abstract

Background: The addition of a FLT3 inhibitor (FLT3i) to standard chemotherapy to treat fit newly diagnosed (ND) patients with *FLT3*-mutated acute myeloid leukemia (AML) represents the standard of care resulting from clinical trial results. However, evidence regarding FLT3i adoption in routine clinical practice is still scarce.

Methods: Clinical data are reported from 394 ND patients with *FLT3*-mutated AML enrolled in the retrospective observational Italian Cohort Study on *FLT3*-mutated patients with AML and treated with an upfront intensive regimen with (FLT3i group, $n = 92$) or without (CT group, $n = 302$) the addition of a FLT3i.

Results: With a median follow-up time of 34.5 months, an effectiveness benefit obtained by FLT3i incorporation both in terms of overall survival (median, 34.9 in the FLT3i vs 12.7 months in the CT group, $p < .01$) and relapse-free survival (median, 18.9 in the FLT3i vs 7.6 months in the CT group, $p = .01$) was documented, with a

higher composite complete remission rate (75.4% in the FLT3i vs 62.4% in the CT group, $p = .052$). FLT3i benefit seemed to be independent from the transplant rate. **Conclusions:** In conclusion, the benefit of FLT3i addition to upfront intensive treatment in newly diagnosed FLT3-mutated AML patients was confirmed in a large, real-life cohort study.

KEYWORDS
acute myeloid leukemia, FLT3 gene mutation, intensive treatment, standard chemotherapy, FLT3 inhibitor, real-life setting

Introduction

Acute myeloid leukemia (AML) is a clonal disorder of hematopoietic stem progenitor cells causing a differentiation block and unregulated proliferation of immature hematopoietic cells.[1] Recently, through the increasing adoption of advanced molecular technologies, a high disease heterogeneity has been elucidated. As a result of the serial acquisition of somatic mutations or specific cytogenetic abnormalities in hematopoietic stem and progenitor cells, the consequent alterations in self-renewal capacity and proliferation control drive neoplastic transformation.[2,3] The identified recurrent genomic abnormalities represent a powerful instrument to stratify AML prognosis and guide therapeutic decisions.[4,5]

Mutations in the FMS-related tyrosine kinase 3 gene (*FLT3*) are some of the most frequent AML genetic alterations, with about one third of adult patients with de novo AML harboring at least a mutation in *FLT3* gene, either internal tandem duplication (ITD) in the juxta-membrane domain or point mutations in the tyrosine kinase domain 2 (TKD), detectable in approximately 25% and 7% of AML cases, respectively.[2,3,6,7] Both mutations are associated with a ligand-independent dimerization of the FLT3 receptor and a constitutive activation of downstream signaling, translating into an aggressive disease phenotype.[8–10] These biological and clinical features make FLT3 an attractive therapeutic target and have led to the development of FLT3 inhibitors (FLT3is). First- (e.g., midostaurin, sorafenib, sunitinib) and second-generation (e.g., gilteritinib, quizartinib, crenolanib) FLT3is have been tested in different settings across several clinical trials. To date, the addition of an FLT3i to intensive chemotherapy to treat fit newly diagnosed patients with *FLT3*-mut AML represents the standard of care. Midostaurin, a type I multitargeted kinase inhibitor active also against KIT, PDGFR, and other receptor tyrosine kinases, combined with cytarabine and daunorubicin (3 + 7) induction and consolidation improved the overall survival in comparison to placebo within phase III RATIFY trial.[11,12] The drug has been the first FLT3i approved in several countries.[13,14] Further studies confirmed its effectiveness benefit with an acceptable safety profile even in patients aged >60 years.[15,16] Recently, the phase III QUANTUM-first clinical trial evaluating quizartinib, a more potent and selective FLT3i, also showed a prolonged overall survival (OS) obtained by adding the drug to standard chemotherapy in comparison to standard chemotherapy plus placebo, with the consequent Food and Drug Administration approval of the drug.[17,18] From these insights, together with ongoing clinical trials evaluating other FLT3is in the same setting, therapeutic alternatives to midostaurin have or may come to light. Nevertheless, results from clinical trials are not always reproducible in the real-life setting, and data regarding the adoption of FLT3is in the first-line treatment of *FLT3*-mut AML in the routine clinical practice are still scarce, with relevant open issues regarding the optimal management of this AML subtype. For instance, the prognostic impact of *FLT3*-ITD mutation has been refined several times according to upcoming evidence, and *FLT3*-TKD mutations represent a distinct biological entity with still a less clear prognostic role.[3,5,19–21] Moreover, the therapeutic algorithm to consolidate *FLT3*-mut patients with AML first remission is debated with significant center-specific differences. In this rapidly evolving scenario, we aimed at investigating the clinical outcomes of the newly diagnosed AML patients with *FLT3*-mut treated in a real-life setting with an upfront intensive treatment and enrolled in the Italian Cohort Study on *FLT3*-mutated AML patients (FLAM).

Methods

Study design and oversight

FLAM (NCT03547258) is an Italian, observational, multicenter cohort study of patients with *FLT3*-mutated AML promoted by IRCCS Istituto Romagnolo per lo studio dei Tumori "Dino Amadori." Clinical and molecular data of patients affected by a *FLT3*-mutated AML were retrospectively and prospectively collected by each participating hematological institution. All patients signed an informed consent form participate in the study and for the treatment of personal data, according to the Declaration of Helsinki, to the ICH Harmonized Tripartite Guideline for Good Clinical Practice, and to Italian laws. No additional procedures, examinations, or blood withdrawal compared to routine practice were performed on enrolled patients. The protocol, informed consent forms, and related documents were

approved by the Ethics Committee of the coordinating center (Ethic Committee of Romagna) in April 2018 (Prot. 3033/2018) and subsequently by the Ethics Committee of each participating institution. No identifiable images were included in the manuscript; therefore, consent for publication was not applicable. The study was supported by Daiichi-Sankyo.

Study population

All consecutive patients treated from January 2012 to June 2021 in the participating centers were considered eligible for inclusion in the FLAM study. Key eligibility criteria were age >18 years old, a confirmed diagnosis of AML according to World Health Organization criteria, and the detection of a positive *FLT3* mutational status (ITD or TKD) either at diagnosis or at disease relapse. Starting from this large Italian cohort of patients with *FLT3*-mutated AML, the present analysis aims to investigate the impact of adding an FLT3i to the upfront intensive AML treatment in a real-life setting. Thus, the subgroup of patients with AML from the overall FLAM population with a documented *FLT3* mutation at diagnosis and receiving intensive chemotherapy as a first line of treatment with or without the incorporation of a FLT3i entered into this analysis. These patients were then divided into FLT3 inhibitor group (FLT3i group) or standard chemotherapy group (CT group). Specifically, each patient who received an FLT3i in addition to intensive chemotherapy in whichever phase of the first line of treatment before either refractoriness, relapse, allogeneic stem cell transplant, or death for any cause has been assigned to FLT3i group.

Outcomes and assessments

Cytogenetic-molecular risk and treatment responses were defined according to the recommendations of the European LeukemiaNet 2022.[5] Particularly, complete remission (CR), complete remission with incomplete recovery (CRi), morphological leukemia-free state, partial remission, and treatment failure (stable disease/progressive disease) were defined based on peripheral blood count and bone marrow blast percentage; timepoints for response assessment were not standardized and defined according to investigator judgment, but information regarding response status were collected after each course of intensive treatment whenever available and in any case of significant changes. Cytogenetic and molecular analyses were performed by each participating center according to its standard policy. Measurable residual disease availability was limited and not homogeneously performed in the study population and thus was not reported in this analysis.

The study endpoints were composite complete remission rate (cCR), which included CR and CRi after induction or reinduction cycles; relapse-free survival (RFS) defined as the time from cCR achievement after induction or reinduction cycles to relapse or death from any cause, whichever occurred first; cumulative incidence of relapse (CIR) defined as the cumulative probability of observing a relapse after cCR achievement as first event respect to death; cumulative incidence of death (CID) defined as the cumulative probability of observing death after cCR achivement as first event respect to relapse; OS, defined as the time from the first day of induction treatment to death from any cause; and event-free survival (EFS), defined as the time from the first day of induction treatment to relapse, death from any cause, or failure to achieve complete remission after induction or reinduction.

The last follow-up update was in October 2021.

Statistical analysis

Overall, data were summarized by median, first (IQ) and third (IIIQ) quartiles for continuous variables and by frequencies and percentages for categorical ones. Chi-squared or Fisher exact tests were used to compare treatment groups with respect to categorical baseline characteristics and response rates. Continuous baseline factors were compared using a *t*-test or Wilcoxon rank sum test, as appropriate. The median follow-up time was computed using the reverse -Meier method and reported with the 95% CIs. The analyses for RFS, CIR, and CID were performed considering only those patients who obtained a CR/CRi within the first line of treatment. The log-rank test was used to compare the Kaplan–Meier curves for RFS, OS, and EFS, whereas the Gray test was used to compare the Aalen–Jonhansen cumulative incidence curves for CIR and CID, respectively.

To investigate the effect of treatment on OS and EFS, Cox proportional hazards model was used. Results were reported as hazard ratios (HRs) and corresponding CIs. To reduce confounding bias originating from structural differences in the two treatment groups concerning prognostic factors, inverse probability weighting was applied. Details regarding this statistical analysis are reported in Supplementary Material.

A mediation analysis was performed to determine whether a specific variable, affected by treatment group, might have had an effect on outcomes that would explain, in whole or in part, the effects documented in each treatment group. To analyze the mediation effect, we compared the effect of treatment on outcome in a model containing only treatment as a covariate (total effect model) against the effect of treatment on outcome in a model containing both treatment and the deemed mediator as covariates (direct effect model). All the details regarding the mediation analysis are reported in Supplementary Material. Exploratory analyses were performed to investigate the effect of treatment on subgroups of patients and to explore the prognostic role of baseline characteristics; to categorize continuous variables, we used the median as the cutoff value.

Two-sided p values <.05 were considered statistically significant.

All analyses were carried out with STATA 15.0 (College Station, Texas, USA) and with R (version 4.2.2). The *ipw* package, version 1.2, of R was used for estimating inverse probability weights.

Results

Patient characteristics

From January 2012 to May 2021, 547 patients with *FLT3*-mutated AML were registered in the FLAM study by 33 Italian participating centers. Data were locked as of October 2021. Overall, 394/547 (72.0%) FLAM-enrolled patients were considered eligible for this analysis. Figure 1 shows details regarding patient selection for this study. Ninety-two of 394 (23.4%) patients received an FLT3 inhibitor in combination with intensive chemotherapy during the first line of treatment (FLT3i group), whereas the remaining 302/394 (76.6%) patients received a standard chemotherapy program (CT group). The two groups were defined as reported in the study population section. Patients in the CT group were treated mainly between 2012 and 2017 (83.0%), whereas those in the FLT3i group were treated between 2018 and 2021 (90.0%). A sensitivity analysis to examine in depth the patients treated with standard chemotherapy after 2018 is reported in Supplementary materials.

Among the study population, the median age at diagnosis was 59 (IQ-IIIQ: 48-66) years and 50% of patients were male. Three-hundred and fifty-eight of 394 (90.9%) patients had a de novo AML, 30/394 (7.6%) a secondary AML, and 6/394 (1.5%) had a therapy-related AML. Regarding *FLT3* mutational status at diagnosis, 341/394 (86.5%) patients harbored an *FLT3*-ITD mutation, 45/394 (11.4%) a TKD mutation, and 5/394 (1.3%) had both mutations. The median allelic ratio (AR) of *FLT3*-ITD was 0.6 (IQ-IIIQ: 0.3-0.9), with an AR $\geq$0.5 in 46/84 (54.8%) patients with available AR at diagnosis. Two-hundred and eighteen of 322 (67.7%) patients with available *NPM1* mutational status at diagnosis harbored a cooccurring *NPM1* gene mutation. The majority of patients with available cytogenetic information at diagnosis had a normal karyotype (260/324, 80.2%), with alterations conferring a favorable prognostic impact in only 2/324 (0.6%) patients, alterations conferring an adverse impact in 16/324 (4.9%), and other alterations in 44/324 (13.6%) patients. Thus, according to available data, 352/373 (94.4%) patients had an intermediate risk AML as defined by ELN22 risk stratification. The median white blood cell count at diagnosis was 44.8 (IQ-IIIQ: 17.0-91.8) $\times$ 10^9/L, with 166/356 (46.6%) patients having a count higher than 50 $\times$ 10^9/L and 81/356 (22.8%) having a count higher than 100 $\times$ 10^9/L. One-hundred and sixty-four patients of the 346 (47.4%) with available information had at least one comorbidity or ongoing clinical complication at study enrollment.

No statistically significant difference between FLT3i and CT group has been observed with respect to the mentioned factors, except for platelet count at diagnosis, which was higher in FLT3i group (median count 64.5 vs 49 $\times$ 10^9/L, $p = .007$). Patients' characteristics are listed in Table 1.

Treatment

The combination of an anthracycline with cytarabine in the so-called "3 + 7" was the most frequently adopted induction regimen in the CT group, performed in 141/302 (46.7%) patients. A three-drug regimen characterized by the addition of etoposide to 3 + 7 backbone was adopted in 102/302 (33.8%) patients. Forty-seven of 302 (15.6%) patients received an intensified fludarabine-containing induction. Last, 12/302 (4.0%) patients received a different induction course.

Among patients in the FLT3i group, 75/92 (81.5%) received an FLT3i in combination intensive chemotherapy starting from induction course, whereas 17/92 (18.5%) patients during subsequent courses of first-line treatment. Specifically, 11/17 (64.7%) received FLT3i during consolidation cycles (10/11 from the first consolidation cycle) and 6/17 (35.3%) patients received the FLT3i as single-agent maintenance therapy to consolidate the obtained response in a pre–hematopoietic stem cell transplantation (HSCT) phase of therapy. Overall, midostaurin was administered in 86/92 (93.5%) patients, whereas only 6/92 (6.5%) patients received sorafenib. Among the 75 patients receiving an FLT3i since induction, the majority of patients received the FLT3i in combination with 3 + 7 (69/75, 92%), 2/75 (2.7%) in combination with high-dose cytarabine, and only 4/75 (5.3%) in combination with a three-drug regimen.

Overall, 10/394 (2.5%) patients received a reinduction course of chemotherapy with the same induction design (eight patients in the standard chemotherapy and two in the FLT3i group) because of suboptimal response to the first induction cycle.

The median number of consolidation cycles in patients achieving a response after induction or reinduction was two (IQ-IIIQ: 1-2) in both groups.

After consolidation chemotherapy cycles, 38/92 (41.3%) patients in the FLT3i group and 83/302 (27.5%) patients in the CT group received an allogeneic stem cell transplantation. Details regarding HSCT donor and stem cell sources, conditioning chemotherapy, and main complications are summarized in Table 2. Only 6/302 (2.0%) patients from the CT group received an autologous stem cell transplant.

Four of 394 (1%) patients received an FLT3i as maintenance therapy after HSCT; sorafenib was the FLT3i administered in every case. Among these patients, only one had already received the FLT3 inhibitor in antecedent phases.

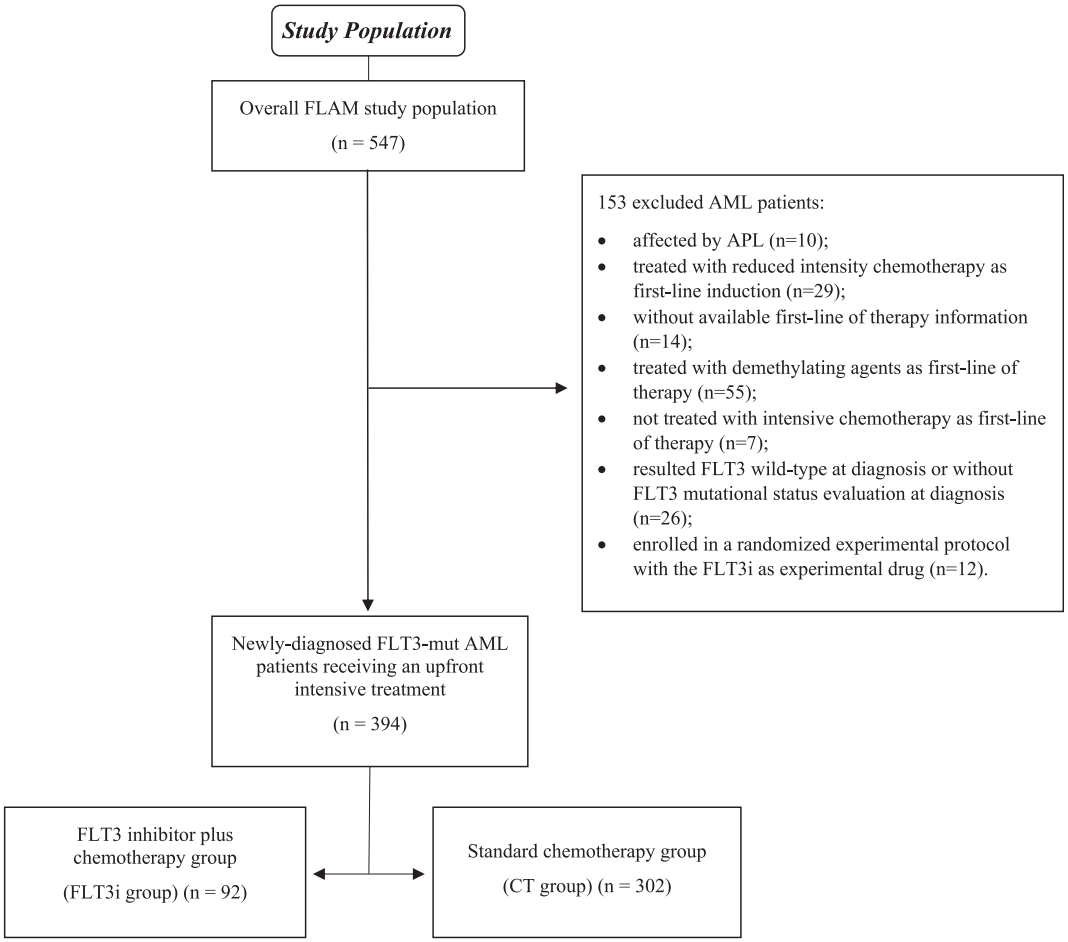

FIGURE 1 Consort diagram: Overall FLAM study population and patient selection for this analysis on patients with newly diagnosed *FLT3*-mutated AML treated intensively. FLAM indicates Italian Cohort Study on *FLT3*-mutated AML patients.

Clinical outcomes

In this study, 70.7% (65/92) and 77.5% (234/302) of patients in the FLT3i and CT groups had adequate information to establish the best overall response after induction or reinduction: the composite complete remission rate (CR + CRi) was 75.4% (49/65) in the FLT3i group and 62.4% (146/234) in the CT group (p = .052). Responses are summarized in Table 3.

Among patients achieving a complete remission, the median RFS was 18.9 months (95% CI, 9.2–not reached) and 7.6 months (95% CI, 5.7–10.2 months) in the FLT3i and CT groups, respectively (p = .01, Figure 2). The CIR was significantly lower in the FLT3i group compared to the CT group (p = .026, Figure 3a). This result did not seem to be the consequence of an increased incidence of death in the FLT3i group, as the CID was not significantly different between the two groups (p = .52, Figure 3b).

Overall, with a median follow-up time of 34.5 months (95% CI, 26.5–43.7 months), the median OS was 34.9 months (95% CI, 16.8–not reached) and 12.7 months (95% CI, 11.6–13.9 months) in the FLT3i and CT groups, respectively (p < .01, Figure 4). The total effect model evaluating the benefit of FLT3i on survival showed that patients in the FLT3i group had a 62% lower hazard of death than patients in the CT group (HR, 0.38; 95% CI, 0.23–0.63), without any baseline difference between the two groups influencing the FLT3i benefit.

After observing a significant difference in survival between the treatment cohorts, we performed a mediation analysis to assess whether a potential disparity in the chance of transplant associated with the treatment group and the transplant-related benefit on survival might have impacted on the FLT3i effect. FLT3i adoption was significantly associated with survival independently of transplant and had an almost identical effect size in both the total effect model and the direct effect model (HR, 0.38; 95% CI, 0.23–0.63). Further analyses regarding transplant and treatment effects on survival are reported in Supplementary Material.

The EFS was 9.4 months (95% CI, 2.9–20.2 months) in the FLT3i group and 3.8 months in the CT group (95% CI, 2.7–5.3 months; p = .003, Figure 5). Patients receiving an FLT3i had 42% lower likelihood of having an event than patients treated only with CT (HR, 0.58; 95% CI, 0.37–0.90). Following the same approach outlined

TABLE 1 Patient characteristics.

Patient characteristics	Overall (*n* = 394)	FLT3i group (*n* = 92)	CT group (*n* = 302)	*p*
Sex, *n* (%)				
Female	196 (50)	42 (46)	154 (51)	
Male	198 (50)	50 (54)	148 (49)	.37
Age at first treatment − year				.88
Median	59	59	59	
IQ-IIIQ	46-65	49-64	47-66	
Range	18-83	23-77	18-83	
N/A	1	/	1	
AML type, *n* (%)				.35
De novo	358 (91)	86 (93)	272 (90)	
Secondary	30 (8)	6 (7)	24 (8)	
Therapy related	6 (1)	/	6 (2)	
FLT3 mutations, *n* (%)				.32
TKD	45 (12)	7 (8)	38 (13)	
ITD	341 (87)	80 (90)	261 (86)	
ITD-TDK	5 (1)	2 (2)	3 (1)	
N/A	3	3	/	
White cell count ($\times 10^9$/L)				.09
Median	44.82	37.04	49.8	
IQ-IIIQ	17.91.83	11.21-77	19.1-93.5	
Range	0.58-525	0.89-280	0.58-525	
N/A	38	3	35	
Platelet count ($\times 10^9$/L)				.007
Median	51	64.5	49	
IQ-IIIQ	34-88	36.5-114.5	31-79	
Range	4-642	10-417	4-642	
N/A	45	4	41	
Neutrophil count, %				.72
Median	10	11.63	10	
IQ-IIIQ	4-25	3-27.2	4.22.8	
Range	0-94.3	0-94	0.94.3	
N/A	168	23	145	
Karyotype, *n* (%)				.18
Favorable	2 (1)	1 (1)	1 (0.4)	
Intermediate	306 (94)	72 (99)	234 (94)	
Adverse	16 (5)	1 (1)	15 (6)	
N/A	70	18	52	
Allelic ratio				.11
Median	0.59	0.4	0.6	
IQ-IIIQ	0.27-0.85	0.26-0.72	0.28-0.91	
Range	0.01-93	0.11-19.5	0.01-93	
N/A	310	63	247	

TABLE 1 (Continued)

Patient characteristics	Overall (n = 394)	FLT3i group (n = 92)	CT group (n = 302)	p
Allelic ratio, *n* (%)				.07
<0.5	38 (45)	17 (59)	21 (38)	
≥0.5	46 (55)	12 (41)	34 (62)	
N/A	310	63	247	
NPM1 status, *n* (%)				.76
Weight	104 (32)	30 (35)	84 (34)	
Mutated	218 (68)	54 (65)	164 (66)	
N/A	62	8	54	
ELN risk, *n* (%)				.87
Favorable	4 (1)	1 (1)	3 (1)	
Intermediate	352 (94)	84 (93)	268 (95)	
Adverse	17 (5)	5 (6)	12 (4)	
N/A	21	2	19	
Ongoing comorbidities, *n* (%)				.19
Yes	164 (47)	31 (41)	133 (49)	
No	182 (53)	45 (59)	137 (51)	
N/A	48	16	32	

Note: Sum of % may not be 100 due to rounding.

Abbreviations: CT group, standard chemotherapy group; ELN, European LeukemiaNet; FLT3i group, FLT3 inhibitor group; ITD, internal tandem duplication; N/A, not available; TKD, tyrosine kinase domain mutation; Wt, wild-type.

previously for OS, we evaluated the impact of HSCT on the EFS benefit: because the direct effect model (HR, 0.62; 95% CI, 0.38–1.02) showed a similar but not identical effect of the FLT3i adoption on EFS compared to the total effect model, we can assume no clear mediation effect of transplant, although a slight influence cannot be excluded.

We additionally analyzed the association between treatment and survival and between treatment and EFS across several subgroups defined by baseline clinical characteristics. The incorporation of FLT3is confirmed their benefit in the different subgroups, albeit not all the associations were statistically significant. All results are reported in Table S1. Investigating whether any of these baseline clinical characteristics might have represented a prognostic factor, older age ($p < .001$; HR for age > 59 vs 1.63; 95% CI, 1.25–2.12) and a higher white cell count ($p = .03$; HR for white cell count >44.82 × 10^9/L 1.37; 95% CI, 1.04–1.81) resulted unfavorable prognostic factors in the overall study population (Table S2).

Discussion

Within FLAM, the addition of an FLT3i to standard chemotherapy improved the outcome of newly diagnosed patients with *FLT3*-mut AML treated in real life with an upfront intensive treatment. In this study portraying the routine clinical practice in the era of advanced clinical development and early availability of FLT3i, the target drug adoption translated into an effectiveness benefit: the OS, as well as EFS and the RFS were longer for patients receiving an FLT3i in comparison to intensive chemotherapy alone; FLT3i benefit on survival did not seem to be mediated by allogeneic stem cell transplant; the target inhibition of the mutated receptor was able to reduce the risk of relapse of a subset of AML historically associated with a consistent risk.

The vast majority of patients receiving an FLT3i in this study were treated as of 2018 and with the multi–tyrosine kinase inhibitor midostaurin; only a few patients received sorafenib. This is in line with midostaurin approval time and that other second-generation FLT3is were still in clinical development during the recruiting period of the FLAM study. Because the study ended in May 2021 with data locked as of October 2021, the follow-up time of the FLT3i group was shorter than that of the CT group, possibly affecting the estimation of treatment effect on clinical outcomes. Because the majority of patients in the CT group were treated before 2018, we have to acknowledge that secular trends of improvements in AML outcome related to the period in which patients have been treated may have an effect on findings. Nevertheless, a sensitivity analysis considering only patients treated after 2018 confirmed the benefit associated with FLT3i adoption.

TABLE 2 Characteristics of allogeneic stem cell transplantation procedures performed to consolidate the first line of treatment of the population involved in this analysis (overall and divided into FLT3i and CT group, respectively).

Allogeneic stem cell transplant details	Overall (n = 121)	FLT3i group (n = 38)	CT group (n = 83)
Response at HSCT, *n* (%)			
cCR (CR + CRi)	109 (99.1)	34 (100.0)	75 (98.7)
PR	1 (0.9)	0	1 (1.3)
N/A	11	4	7
Donor type, *n* (%)			
HLA-matched sibling donors	26 (21.5)	10 (26.3)	16 (19.2)
HLA-matched unrelated donors	44 (36.4)	18 (47.4)	26 (31.3)
HLA-haploidentical donors (haplo)	48 (39.7)	9 (23.7)	39 (47)
Umbilical cord bloods	3 (2.5)	1 (2.6)	2 (2.4)
Stem cell source, *n* (%)			
PBSC	80 (74.8)	31 (91.2)	49 (67.1)
BM	24 (22.4)	2 (5.9)	22 (30.1)
CORD	3 (2.8)	1 (2.9)	2 (2.7)
N/A	14	4	10
Conditioning intensity, *n* (%)			
RIC	14 (12.3)	8 (22.2)	6 (7.7)
FULL	93 (81.6)	25 (69.4)	68 (87.2)
Other	7 (6.1)	3 (8.3)	4 (5.1)
N/A	7	2	5
GVHD, *n* (%)			
Any grade GVHD	41 (39.0)	15 (44.1)	26 (36.6)
Grade I-II	35 (33.3)	14 (41.2)	21 (29.6)
Grade III-IV	6 (5.7)	1 (2.9)	5 (7.0)
No GVHD	64 (61.0)	19 (55.9)	45 (63.4)
N/A	16	4	12

Note: Sum of % may not be 100 due to rounding.

Abbreviations: BM, bone marrow; cCR, composite complete response; CR, complete remission; CRi, complete remission with incomplete recovery; CT group, standard chemotherapy group; FLT3i group, FLT3 inhibitor group; GVHD, graft versus host disease; HSCT, hematopoietic stem cell transplantation; N/A, not available; PBSC, peripheral blood stem cells; PR, partial response; RIC, reduced-intensity conditioning regimen.

Despite this study confirming the benefit associated with an FLT3i addition to standard chemotherapy obtained in different experimental studies, the median OS for each treatment group seems to be sensitive lower if compared to that registered in the milestone phase 3 RATIFY trial (CALGB 10603), evaluating midostaurin in combination with standard chemotherapy in newly diagnosed FLT3-mutated AML patients (34.9 and 12.7 months for the FLT3i and CT group in the present study; 74.7 and 25.6 months for the midostaurin and the placebo group in RATIFY trial).[12] First, results obtained in clinical trials are not always reproducible in real life. Moreover, among the factors potentially influencing this difference, the median age of our study population (59 years for both FLT3i and CT group, respectively) was clearly higher in comparison to the mentioned trial (47.1 and 48.6 years, respectively) and older age (>59 years) resulted an unfavorable prognostic factor in our study, as expected. For instance, in the AMLSG 16-10 single-arm phase II clinical trial specifically designed to also include older patients (up to 70 years) and with a median age of the study population more similar to ours (median age, 54.1 and 50.5 in the experimental arm and selected historical controls, respectively) the documented survival rates were also closer (median OS of 36.2 months for patients who received midostaurin and 13.2 months for the historical controls treated with chemotherapy alone).[16,22] Of note, the estimated HR for death in this study was very low (HR FLT3i vs. CT 0.38) in comparison to the previously mentioned studies. Some factors may have highly influenced this result. First, we reported a lower early death (ED) rate in the FLT3i group (1.5%) in comparison to the CT group (5%) and to other reported ED rates associated with this therapeutic regimen (e.g., the ED rate was 5.9% in the FLT3i group and 4.8% in the CT group of

TABLE 3 Best overall responses to standard chemotherapy plus FLT3 inhibitor or standard chemotherapy alone documented after induction or reinduction, whenever administered.

BOR after induction or reinduction	FLT3i group (n = 92)	CT group (n = 302)
CR	46 (70.8%)	144 (61.5%)
CRi	3 (4.6%)	2 (0.9%)
PR	2 (3.1%)	22 (9.4%)
SD/HI	/	1 (0.4%)
SD	7 (10.8%)	19 (8.1%)
PD	6 (9.2%)	34 (14.5%)
ED	1 (1.5%)	12 (5.1%)
N/A	27	68

Note: Sum of % may not be 100 due to rounding.

Abbreviations: CR, complete remission; Cri, complete remission with incomplete recovery; CT group, standard chemotherapy group; ED, early death in 30 days from treatment start; FLT3i group, FLT3 inhibitor group; HI, hematologic improvement; N/A, not available; PD, progressive disease; PR, partial response; SD, stable disease.

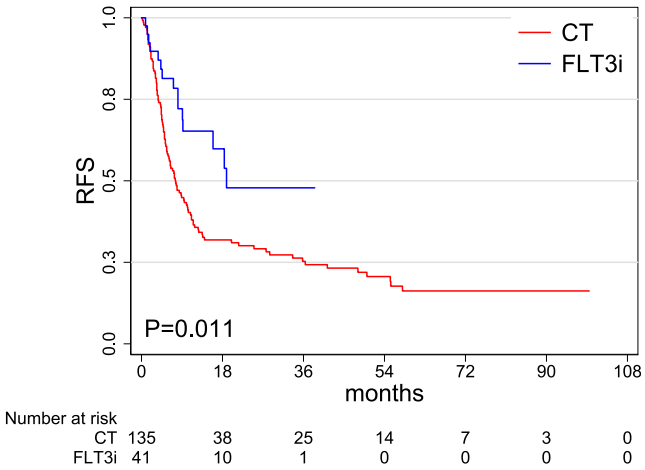

FIGURE 2 Kaplan–Meier estimates of relapse-free survival for patients receiving an FLT3 inhibitor in addition to a standard chemotherapy program and for patients receiving standard chemotherapy alone.

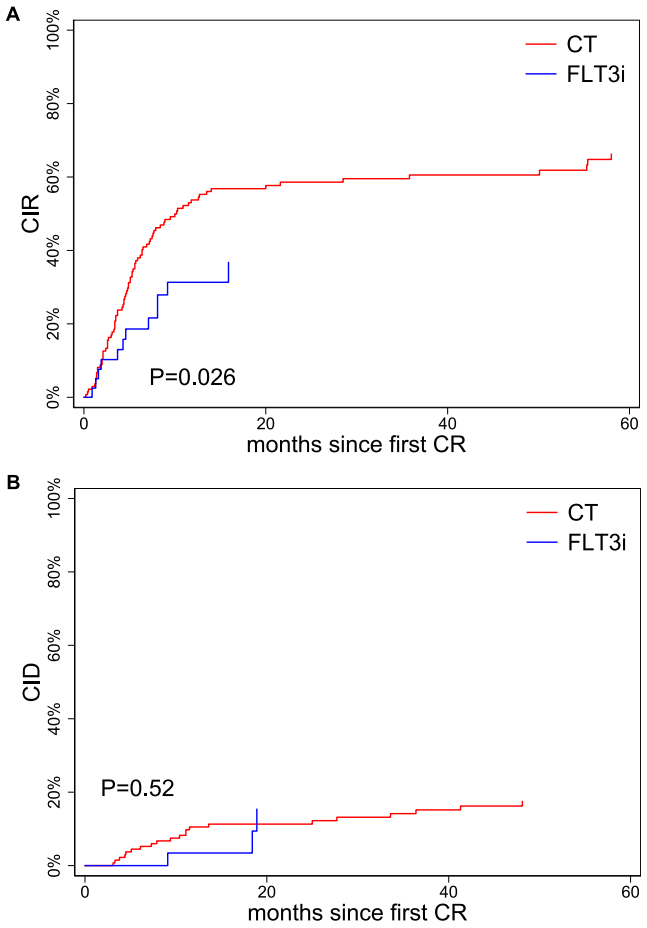

FIGURE 3 Aalen–Johansen estimate of (a) cumulative incidence of relapse (CIR) between the different treatment groups and (b) cumulative incidence of death (CID) after the achievement of complete remission between the different treatment groups.

Dohner et al[16]). Second, it has been reported in the same study there was a more pronounced OS benefit associated with the addition of an FLT3i in older patients. This difference registered even in our study (HR FLT3i vs. CT age ≤59; 0.46; 95% CI, 0.23–0.94; HR FLT3i vs. CT age >59; 0.27; 95% CI, 0.12–0.60). This last factor may have a strong impact on the HR for death in this study considering that the median age of our study population is clearly higher, as mentioned. However, several differences in inclusion criteria and treatment plans have to be considered as sensitive limitations of comparisons with other studies. In particular, in the CT group of the present study, about half of the patients received a different-from 3 + 7 induction therapy, with either the addition of a third agent (etoposide or fludarabine) or including high-dose cytarabine. This heterogeneity has also to be taken into account in evaluating efficacy outcomes documented in the CT group, according to available data suggesting potential improvements associated with the intensification.[23–26]

HSCT has been generally considered the recommended consolidation therapy for patients with *FLT3*-ITD mutated AML in first complete remission for the procedure.[1,27–29] Nevertheless, in the past, reported evidence highlighted how HSCT benefit might have been restricted to patients with a high allelic ratio of *FLT3*-ITD and those with a favorable ELN17 risk might have not required a transplant as consolidation.[4,30–32] In our study, with different risk classification systems and center-specific policies modifications occurring over time, a high adoption of allogeneic stem transplant has been documented (the 69.4% and 51.4% obtaining a cCR in our study underwent HSCT in the FLT3i and CT group, respectively) to consolidate the achievement of CR. A mediation analysis considering HSCT as a potential mediator of the association between FLT3i and survival, particularly relevant because the previously reported lower transplant rate in the CT group in comparison to the FLT3i group showed that FLT3i benefit on survival seemed to be independent from the transplant rate. The systematic unavailability of measurable

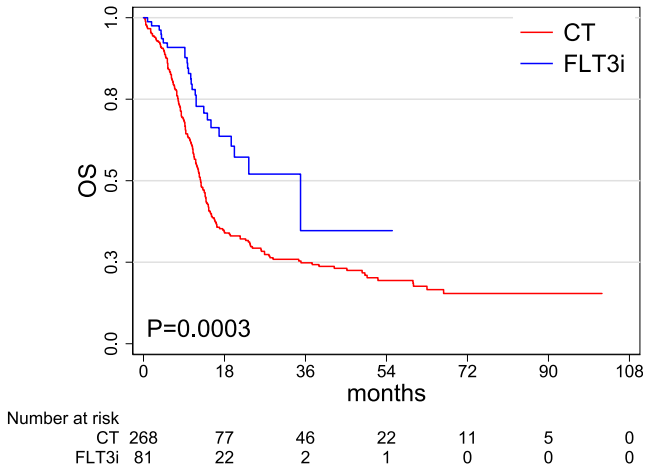

FIGURE 4 Kaplan–Meier estimates of survival for patients receiving an FLT3 inhibitor in addition to a standard chemotherapy program and for patients receiving standard chemotherapy alone.

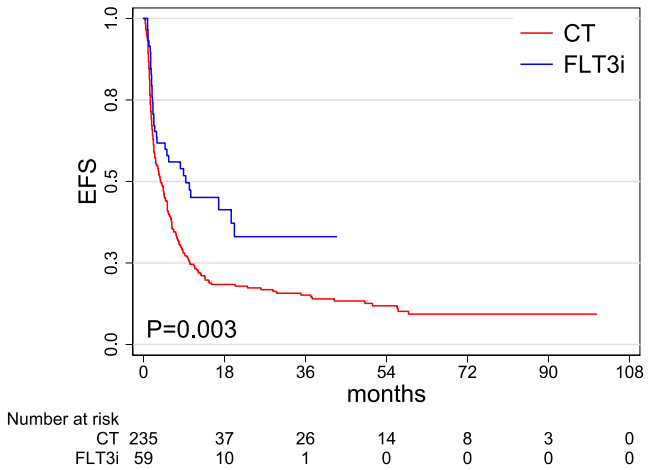

FIGURE 5 Kaplan–Meier estimates of event-free survival for patients receiving an FLT3 inhibitor in addition to a standard chemotherapy program and for patients receiving standard chemotherapy alone.

residual disease data and the incomplete genomic characterization of several involved patients limit the possibility of this study to evaluate the role of HSCT in the current therapeutic paradigm, as well as the lack of next-generation sequencing data limits the possibility to properly define AML risk with the current and updated ELN2022 risk stratification for the majority of cases.[5] Future studies specifically addressing the role of cooccurring mutations and minimal residual disease clearance in determining the HSCT indication of *FLT3*-mut AMLs are awaited. Unsurprisingly, the rate of adoption of an autologous stem cell transplant and of a maintenance therapy with an FLT3i after HSCT were low, the latter probably from a limited access to the drug in that setting in our country.

In conclusion, with all the limitations of a retrospective and observational study, this analysis confirms the benefit of a FLT3i incorporation into the upfront intensive treatment of *FLT3*-mut AML in a large cohort of Italian patients with AML treated in the past decade. In a context where the recent release of quantum-first data stated quizartinib as a potential treatment option for patients with ND *FLT3*-ITD mut AML and other clinical trials evaluating different FLT3i in this setting are ongoing, these data provide useful information to properly evaluate FLT3i performances in the real-life setting and measure upcoming evidences.

AUTHOR CONTRIBUTIONS

Jacopo Nanni: Conceptualization; Methodology; Writing - original draft; Writing - review & editing; Investigation; Validation; and Visualization. **Irene Azzali**: Conceptualization; Methodology; Writing - original draft; Writing - review & editing; Data curation; Formal analysis; Software; Validation; and Visualization. **Cristina Papayannidis**: Conceptualization and Investigation. **Antonino Mulè**: Conceptualization and Investigation. **Ernesta Audisio**: Conceptualization and Investigation. **Maria Paola Martelli**: Conceptualization and Investigation. **Barbara Scappini**: Conceptualization and Investigation. **Patrizia Chiusolo**: Conceptualization and Investigation. **Benedetta Cambò**: Conceptualization and Investigation. **Anna Candoni**: Conceptualization and Investigation. **Monia Lunghi**: Conceptualization and Investigation. **Francesco Albano**: Conceptualization and Investigation. **Attilio Olivieri**: Conceptualization and Investigation. **Nicola Fracchiolla**: Conceptualization and Investigation. **Massimo Bernardi**: Conceptualization and Investigation. **Claudio Romani**: Conceptualization and Investigation. **Gian Matteo Rigolin**: Conceptualization and Investigation. **Maria Benedetta Giannini**: Conceptualization and Investigation. **Monica Bocchia**: Conceptualization and Investigation. **Elisabetta Todisco**: Conceptualization and Investigation. **Daniela Cilloni**: Conceptualization and Investigation. **Maria Teresa Bochicchio**: Conceptualization and Investigation. **Emanuela Ottaviani**: Conceptualization and Investigation. **Agnese Mattei**: Conceptualization and Investigation. **Federica Zamagni**: Conceptualization and Investigation. **Irene Valli**: Conceptualization and Data curation. **Roberta Volpi**: Conceptualization and Data curation. **Giovanni Marconi**: Conceptualization; Methodology; Writing - review & editing; and Investigation. **Elisabetta Petracci**: Conceptualization; Methodology; Writing - review & editing; Software and Formal analysis. **Giovanni Martinelli**: Conceptualization; Methodology; Writing - review & editing; Funding acquisition; Project administration; Resources; Validation and Supervision. **Francesco Lanza**: Conceptualization. **Annamaria Mianulli**: Conceptualization. **Bianca Serio**: Conceptualization. **Angelo Michele Carella**: Conceptualization. **Daniele Vallisa**: Conceptualization. **Nicola Di Renzo**: Conceptualization. **Federica Gigli**: Conceptualization. **Matteo Carrabba**: Conceptualization. **Irene Urbino**: Conceptualization. **Carlotta Zavatto**: Conceptualization. **Clara De Ambrogi**: Conceptualization. **Alessandro Cignetti**: Conceptualization. **Beatrice Sani**: Conceptualization. **Esther Oliva**: Conceptualization. **Valentina Robustelli**: Conceptualization. **Sabrina Crivellaro**: Conceptualization. **Roberto Bono**: Conceptualization. **Monica Rizzo**: Conceptualization. **Matteo Piccini**: Conceptualization. **Sofia Pilerci**: Conceptualization. **Alberto Bosi**: Conceptualization. **Matteo Molica**: Conceptualization. **Simona Sica**: Conceptualization. **Valeria Cardinali**: Conceptualization. **Chiara Cantò**: Conceptualization. **Giuseppe Pietrantuono**: Conceptualization. **Fabrizio Pane**: Conceptualization. **Amelia Rinaldi**: Conceptualization.

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CONFLICT OF INTEREST STATEMENT

GM: Consultant/speaker bureau of Abbvie, Astellas, AstraZeneca, Immunogen, Jansenn, Menarini/Stemline, Pfizer, Ryvu, Servier, Syros, and Takeda; research support from Abbvie, Astellas, AstraZeneca, Daiichi Sankyo, Pfizer, and Syros. CP: Honoraria of Astellas and Novartis, Advisory Board of Astellas and Novartis. All other authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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