Oncol Ther
https://doi.org/10.1007/s40487-026-00427-w

ORIGINAL RESEARCH

Gain and Loss of *FLT3* Mutations in Patients with Acute Myeloid Leukemia: A Noninterventional Cohort Study (CLEVO)

Cristina Papayannidis · Irina Amitai · Pascal Turlure · Ann De Becker · Felix Mensah · Dina Elsouda · Ioanna Vardounioti · Jose Alejandro Palacios-Fabrega · Paresh Vyas · Blanca Boluda

Received: November 6, 2025 / Accepted: February 17, 2026

ABSTRACT

Introduction: *FMS*-like tyrosine kinase 3 (*FLT3*) mutations show variable detectability in relapse/refractory acute myeloid leukemia (AML) with unclear clonal evolution dynamics.

Methods: This prospective noninterventional study examined clonal evolution and outcomes from AML diagnosis to relapse/refractory disease occurrences.

Results: Of 650 patients included, 172 were *FLT3*-positive (*FLT3*^{pos}) and 472 were *FLT3*-negative (*FLT3*^{neg}; 99.1% testing rate; unknown *FLT3* status, six patients). At first occurrence, the *FLT3* testing rate decreased (57.0% [166/291]). Among tested patients, 45 had *FLT3*^{pos} and 121 had *FLT3*^{neg} AML. A gain or loss of mutations was seen in 15.6% (7/45) of patients with *FLT3*^{pos} AML and 14.9% (18/121) of patients with *FLT3*^{neg} AML. Median (95% confidence interval [CI]) overall survival was 22.8 (19.6, not estimable [NE]) months across patients (*FLT3*^{pos}, NE;*FLT3*^{neg}, 20.3 [15.2–23.7] months; hazard

Prior Presentation: This manuscript is based on work that was previously presented at the American Society of Hematology Annual Meeting (10–13 December 2022; New Orleans, LA, USA) and the European Haematology Association Annual Meeting (13–16 June 2024; Madrid, Spain).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40487-026-00427-w.

C. Papayannidis
IRCCS Azienda Ospedaliero Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Bologna, Italy

I. Amitai
Sheba Medical Center, Tel Aviv University, Tel Aviv, Israel

P. Turlure
Limoges University Hospital, Limoges, France

A. De Becker
Universitair Ziekenhuis Brussel, Vrije Universiteit, Brussels, Belgium

F. Mensah
Indiana Blood and Marrow Transplant, Indianapolis, IN, USA

D. Elsouda
Astellas Pharma Inc., Northbrook, IL, USA

I. Vardounioti · J. A. Palacios-Fabrega
Astellas Pharma Europe Ltd., Addlestone, UK

P. Vyas (✉)
University of Oxford, Oxford, UK
e-mail: paresh.vyas@imm.ox.ac.uk

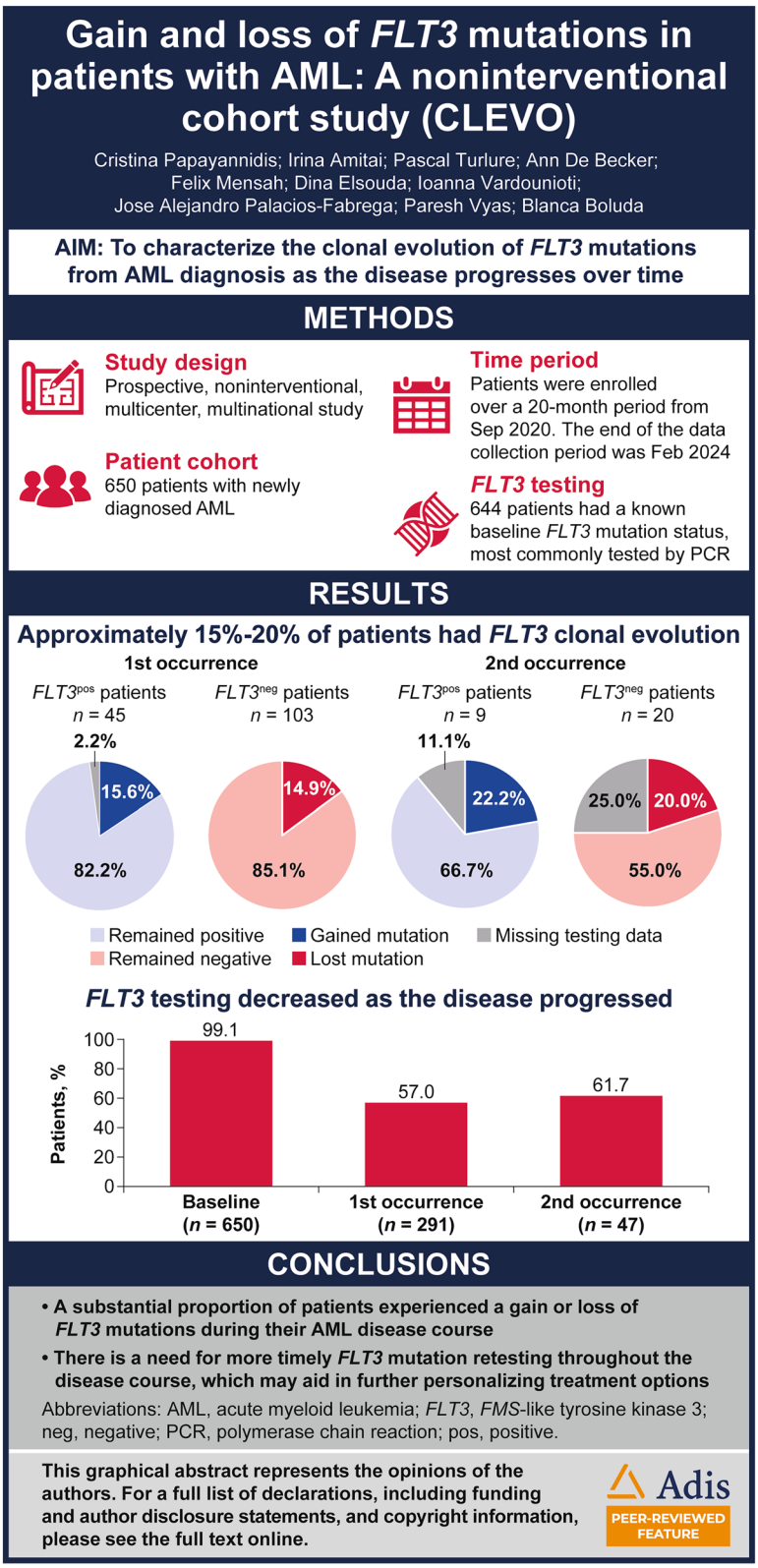
B. Boluda
Hematology Department, La Fe University Hospital, Valencia, Spain

Published online: 11 March 2026

ratio [HR] [95% CI] 0.6 [0.5–0.8]). Median (95% CI) disease-free survival across patients was NE (26.3–NE) (*FLT3*^{pos}, NE;*FLT3*^{neg}, NE; HR [95% CI] 0.8 [0.6–1.1]). Median event-free survival (95% CI) was 11.8 (10.0–15.5) months in all patients (*FLT3*^{pos}, 17.2 [11.0–NE] months;*FLT3*^{neg}, 10.4 [8.4–13.2] months; HR [95% CI] 0.8 [0.6–1.0]).

***Conclusions*:** Dynamic changes in *FLT3* mutation status were observed during these patients' disease course. *FLT3*^{pos} status at baseline, but not at first occurrence, was associated with improved outcomes; other confounders should be considered. Timelier *FLT3* mutation retesting may aid in personalizing treatment. Graphical abstract available for this article.

Graphical Abstract:



Keywords: AML; Clonal evolution; Mutation detection; Tyrosine kinases; *FLT3* mutations; Relapsed/refractory disease

Key Summary Points
Why carry out this study?
Patients with acute myeloid leukemia (AML) carrying *FMS*-like tyrosine kinase 3 (*FLT3*) mutations display clonal evolution over the course of their disease.
This study intended to quantify clonal evolution of *FLT3* mutations in patients with AML, and to identify factors associated with clonal evolution.
Patient survival was also evaluated in terms of clonal evolution.
What was learned from the study?
At first occurrence of relapsed/refractory disease, *FLT3* testing was not consistently performed; however, approximately 15% of patients tested had gained or lost *FLT3* mutations, highlighting the need for retesting.
A deeper understanding of *FLT3* clonal evolution and its clinical consequences in AML is needed to optimize treatment for patients with *FLT3*^{pos} AML.

DIGITAL FEATURES

This article is published with digital features, including a graphical abstract, to facilitate understanding of the article. To view digital features for this article, go to https://doi.org/10.6084/m9.figshare.31347682.

INTRODUCTION

Mutations in the *FMS*-like tyrosine kinase 3 (*FLT3*) gene are some of the most common in acute myeloid leukemia (AML). They are found in approximately one-third of patients [1], most commonly as internal tandem duplications (ITD) (*FLT3*-ITD, 19–25% [2, 3] of adult patients), or tyrosine kinase domain point mutations (TKD) (*FLT3*-TKD, 5–6% of adult patients) [3, 4]. Frequency of *FLT3* mutations decreases with age after adulthood [2, 3]. Nevertheless, a substantial proportion of older patients with AML have *FLT3* mutations [3, 5].

FLT3-ITD mutations are associated with poorer clinical outcomes [6, 7]. The prognostic impact of *FLT3*-TKD mutations is unclear; however, they have been associated with acquired resistance to FLT3 inhibitors [8, 9]. *FLT3* mutations often arise as secondary events in leukemic transformation [10] and may not be consistently present throughout the disease course [11]; thus, testing is important at every disease stage.

Currently, genetic testing is recommended at diagnosis for all patients with AML to identify targeted therapies [12], and should be repeated at relapse or disease progression, highlighting the potential of clonal evolution [12]. Despite this recommendation and the availability of FLT3 inhibitors [12, 13], *FLT3* testing is still not consistently performed at relapse or disease progression [14]. Moreover, the impact of *FLT3* clonal evolution is poorly understood, and a better understanding of its dynamics and clinical consequences is necessary to aid in optimal treatment selection for patients with *FLT3*-mutated AML. This prospective study aimed to characterize *FLT3* clonal evolution from AML diagnosis through disease progression.

METHODS

Ethics

This study adhered to International Council for Harmonisation guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable regulations. A full list of all institutional ethics committee (IEC)/institutional review board (IRB) approval dates and numbers is provided in Supplementary Table 1. Written informed consent was obtained from all patients. The protocol and relevant documents were approved by the IRBs/IECs at each study

site. Data were analyzed by the sponsor, with all authors having access to the study data.

Study Design

This was a prospective, noninterventional study of the clonal evolution of *FLT3* mutations during disease progression in patients with newly diagnosed AML (CLEVO) recruited from 57 oncology or hematology/oncology specialist sites across Europe (Belgium, France, Germany, Italy, Spain, and the UK), Israel, and the USA (Fig. 1). Patients were enrolled over 20 months starting from September 2020 with a planned 3-year follow-up from the date of informed consent until early discontinuation or death, whichever occurred first.

Patients were treated according to the local standard of care at the investigator's discretion. Two interim analyses were planned at approximately 1 and 3 years after the study initiation. Following the second interim analysis [15], the study was terminated by the sponsor because patient enrollment was complete, and 43.8% ($n = 258/589$) of patients had experienced a first occurrence event (defined as relapse and/or refractory event) to treatment with an *FLT3* testing rate of 97.7%. Only 89 patients were alive with a record of a first occurrence, and there was a lower *FLT3* testing rate and patient survival from the second occurrence onward. As a result, it was determined that continuing the study would not contribute significantly to the assessment of the primary endpoint. Thus, the data collection period ended on 16 February 2024 instead of 15 May 2025.

Patients

Eligible patients were adults (≥ 18 years) who received an AML diagnosis according to the World Health Organization (WHO) 2016 classification [16]. Additional inclusion and exclusion criteria and genetic and cytogenetic testing methods are listed in the Supplementary Methods.

Objectives and Endpoints

The primary objective was to determine *FLT3* clonal evolution in patients with AML throughout their disease course, defined as the proportion of patients with *FLT3* mutation-positive ($FLT3^{pos}$) AML, which was previously negative ($FLT3^{neg}$), or vice versa. The primary endpoint was the proportion of patients with *FLT3* clonal evolution assessed at consecutive occurrences.

Secondary objectives were to describe *FLT3* mutations, cytogenetic abnormalities, other AML mutations, patient survival, and composite complete remission (CRc). Secondary endpoints and post hoc analyses are described in the Supplementary Methods and Supplementary Table 2.

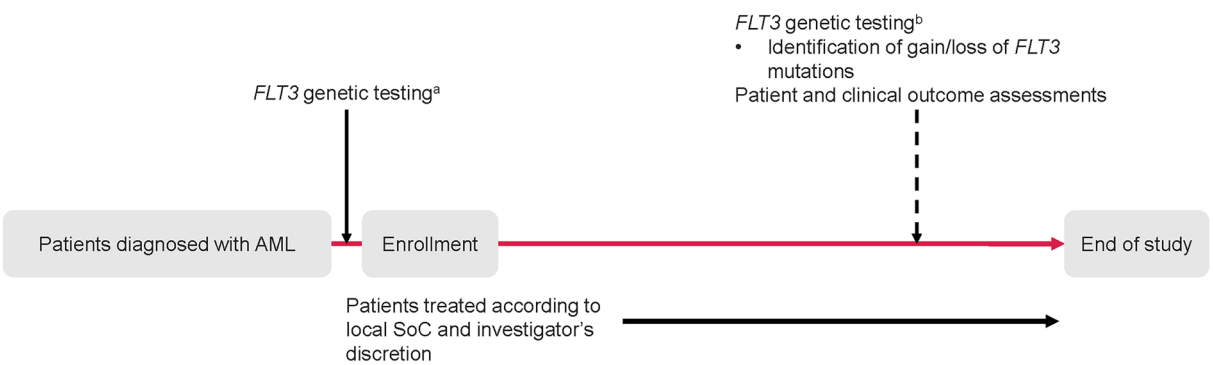


Fig. 1 Study design. [a]Genetic testing was performed prior to the initiation of AML treatment. [b]Genetic testing was performed at relapse or refractory events. *AML* acute myeloid leukemia, *FLT3 FMS*-like tyrosine kinase 3, *SoC* standard of care

Statistical Analyses

This study was descriptive without prespecified statistical hypotheses. No formal statistical testing was performed, and no *p*-values are reported. All comparisons were exploratory. All endpoints were analyzed using the full analysis set (FAS; all patients with ≥ 1 data point after enrollment). For the primary and secondary endpoint analysis, baseline period was defined as the period from the first assessment at enrollment until first occurrence. Baseline values corresponded to the value of assessment at enrollment. Additional details on sample size calculations, statistical analyses, and post hoc analyses are provided in the Supplementary Methods.

RESULTS

Patient Disposition

In total, 675 patients signed informed consent forms; of those, 650 were enrolled and included in the FAS, and 25 failed screening (Supplementary Fig. 1). A total of 12 patients (1.8%) completed the 3-year follow-up. The most common reasons for study discontinuation were death (327/650 [50.3%]) and early termination of the study owing to sponsor decision (286/650 [44.0%]).

Baseline Characteristics and Demographics

At baseline (i.e., period from enrollment to first occurrence), 172/650 (26.5%) patients had *FLT3*^{pos} AML, 472/650 (72.6%) had *FLT3*^{neg} AML, and 6/650 (0.9%) had an unknown *FLT3* status (Table 1). The median (range) age for patients with *FLT3*^{pos} and *FLT3*^{neg} AML was 59.0 (22–87) years and 67.0 (19–93) years, respectively. Most (481/650 [74.0%]) patients had de novo AML. Intermediate and adverse risk patients made up 38.4% (66/172) and 15.1% (26/172) of patients with *FLT3*^{pos} AML, respectively, and 30.7% (145/472) and 34.3% (162/472) of patients with *FLT3*^{neg} AML, per European LeukemiaNet (ELN) 2017 [17] criteria. Of 172 patients with *FLT3*^{pos} AML, 124 (72.1%) had *FLT3*-ITD, and 49 (28.5%) had *FLT3*-TKD.

AML Treatment Received at Baseline

Among those with known mutational status, the most common treatment during baseline was high-intensity chemotherapy alone (*FLT3*^{pos}, 48/172 [27.9%]; *FLT3*^{neg}, 193/472 [40.9%]; total, 241/644 [37.4%]). In patients with *FLT3*^{pos} and *FLT3*^{neg} AML, high-intensity chemotherapy plus FLT3 inhibitors (*FLT3*^{pos}, 67/172 [39.0%]; *FLT3*^{neg}, 0/472 [0%]; total, 67/650 [10.4%]) and hypomethylating agent (HMA) plus venetoclax (*FLT3*^{pos}, 17/172 [9.9%]; *FLT3*^{neg}, 101/472 [21.4%]; total, 119/644 [18.5%]) were the second most common, respectively (Supplementary Table 3). Overall, 54/644 (8.4%) patients underwent a hematopoietic stem cell transplantation (*FLT3*^{pos}, 18/172 [10.5%]; *FLT3*^{neg}, 36/472 [7.6%]).

FLT3 Testing

FLT3 testing rates decreased with each occurrence. In total, 291 patients had a first occurrence, of which 166 (57.0%) had *FLT3* testing at baseline; at second occurrence ($n = 47$), *FLT3* testing rate was 61.7% (29/47). Polymerase chain reaction (PCR) was the most common *FLT3* testing method at baseline (72.9% [474/650]) and at first occurrence (41.6% [121/291]). Next-generation sequencing (NGS) was used in 19.2% (125/650) and 4.5% (13/291) of patients at baseline and first occurrence, respectively. A small proportion of patients were tested with other, not specified methods (Table 2).

Clonal Evolution

At first occurrence, 45/166 (27.1%) and 121/166 (72.9%) patients had *FLT3*^{pos} and *FLT3*^{neg} AML, respectively. Of 45 patients with *FLT3*^{pos} AML, 1 had missing *FLT3* testing data at first occurrence, 37 (82.2%) were previously *FLT3*^{pos}, and 7 (15.6%) were previously *FLT3*^{neg} and thus gained a mutation (Fig. 2). In total, 32 patients had *FLT3*-ITD AML at first occurrence; of these,

Table 1 Patient baseline characteristics (FAS)

Characteristic	*FLT3*^{pos} (*n* = 172)	*FLT3*^{neg} (*n* = 472)	Unknown/missing (*n* = 6)	Total (*n* = 650)
Sex, *n* (%)				
Female	84 (48.8)	205 (43.4)	1 (16.7)	290 (44.6)
Male	88 (51.2)	267 (56.6)	5 (83.3)	360 (55.4)
Age, years				
Mean (SD)	58.8 (15.2)	63.9 (14.5)	78.8 (8.8)	62.7 (14.9)
Median (range)	59.0 (22–87)	67.0 (19–93)	77.5 (67–94)	64.5 (19–94)
Country, *n* (%)				
Belgium	14 (8.1)	31 (6.6)	0	45 (6.9)
France	30 (17.4)	43 (9.1)	0	73 (11.2)
Germany	4 (2.3)	15 (3.2)	0	19 (2.9)
Israel	13 (7.6)	69 (14.6)	1 (16.7)	83 (12.8)
Italy	49 (28.5)	161 (34.1)	1 (16.7)	211 (32.5)
Spain	36 (20.9)	114 (24.2)	4 (66.7)	154 (23.7)
UK	22 (12.8)	32 (6.8)	0	54 (8.3)
USA	4 (2.3)	7 (1.5)	0	11 (1.7)
AML diagnosis, *n* (%)				
De novo	151 (87.8)	327 (69.3)	3 (50.0)	481 (74.0)
Secondary AML	20 (11.6)	145 (30.7)	3 (50.0)	168 (25.8)
Missing	1 (0.6)	0	0	1 (0.2)
Diagnosis method, *n* (%)				
Bone marrow	147 (85.5)	439 (93.0)	5 (83.3)	591 (90.9)
Blood smear	24 (14.0)	33 (7.0)	1 (16.7)	58 (8.9)
Missing	1 (0.6)	0	0	1 (0.2)
ELN 2017 risk category, *n* (%)				
Favorable	40 (23.3)	94 (19.9)	0	134 (20.6)
Intermediate	66 (38.4)	145 (30.7)	2 (33.3)	213 (32.8)
Adverse	26 (15.1)	162 (34.3)	3 (50.0)	191 (29.4)
Not available	38 (22.1)	70 (14.8)	1 (16.7)	109 (16.8)
Missing	2 (1.2)	1 (0.2)	0	3 (0.5)
FLT3 mutations[a], *n* (%)				
ITD	124 (72.1)	N/A	N/A	124 (19.1)

Table 1 continued

Characteristic	*FLT3*^{pos} (*n* = 172)	*FLT3*^{neg} (*n* = 472)	Unknown/missing (*n* = 6)	Total (*n* = 650)
TKD	49 (28.5)	N/A	N/A	49 (7.5)
Other *FLT3* mutations	11 (6.4)	N/A	N/A	11 (1.7)
Cytogenetics, *n*/*N* (%)				
Not assessed	13/172 (7.6)	18/472 (3.8)	0	31/650 (4.8)
Normal karyotype	112/169 (66.3)	205/461 (44.5)	1/2 (50.0)	318/632 (50.3)
t (8;21)	5/169 (3.0)	12/461 (2.6)	0	17/632 (2.7)
t (15;17)	1/169 (0.6)	1/461 (0.2)	0	2/632 (0.3)
Inv (16)	5/169 (3.0)	17/461 (3.7)	0	22/632 (3.5)
t (16; 16)	0	2/461 (0.4)	0	2/632 (0.3)
Monosomy	4/169 (2.4)	34/461 (7.4)	0	38/632 (6.0)
Complex karyotype	8/169 (4.7)	96/461 (20.8)	1/2 (50.0)	105/632 (16.6)
Other	26/169 (15.4)	104/461 (22.6)	0	130/632 (20.6)
Missing	3/172 (1.7)	11/472 (2.3)	4/6 (66.7)	18/650 (2.8)

[a]Patients may have tested positive for > 1 *FLT3* mutation

AML acute myeloid leukemia, *ELN* European LeukemiaNet, *FAS* full analysis set, *FLT3 FMS*-like tyrosine kinase 3, *inv* inversion, *ITD* internal tandem duplication, *N/A* not available, *neg* negative, *pos* positive, *SD* standard deviation, *TKD* tyrosine kinase domain point mutation

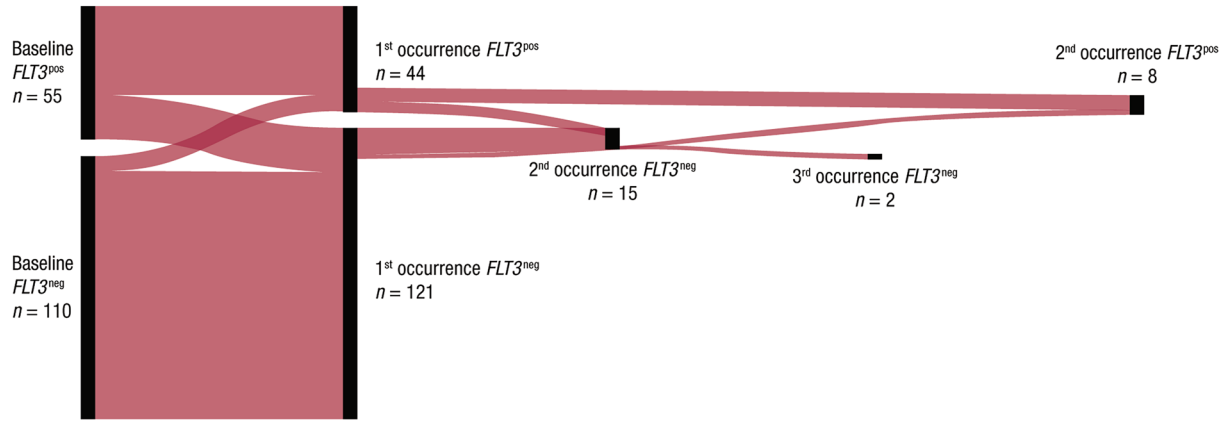
31 retained their *FLT3*-ITD mutations from baseline, 1 patient lost their *FLT3*-ITD mutation but gained a *FLT3*-TKD mutation, and 1 patient retained their *FLT3*-ITD mutation but also gained a *FLT3*-TKD mutation. Seven patients had *FLT3*-TKD mutations at first occurrence; of these, 5 patients retained their *FLT3*-TKD mutations from baseline, 2 patients lost their *FLT3*-TKD mutations, but gained a *FLT3*-ITD mutation, 1 patient retained their *FLT3*-TKD mutation and gained a *FLT3*-ITD mutation, and 1 patient gained another type of *FLT3* mutation in addition to their *FLT3*-TKD mutation. Of the 7 patients with *FLT3*^{neg} AML at baseline who gained a *FLT3* mutation at first occurrence, all gained *FLT3*-ITD mutations. Of 121 patients with *FLT3*^{neg} AML at baseline, 103 (85.1%) remained *FLT3*^{neg} and 18 (14.9%) were previously *FLT3*^{pos} (*FLT3*-ITD, *n* = 18; *FLT3*-TKD, *n* = 17; other *FLT3* mutations, *n* = 17; counts not mutually exclusive as patients may have had more than 1 type of *FLT3* mutation) and thus lost a mutation. Of 29 patients with a second occurrence and *FLT3* testing, 9 and 20 were *FLT3*^{pos} and *FLT3*^{neg}, respectively. Of 9 patients with *FLT3*^{pos} AML, 1 had missing *FLT3* testing data, 6 (66.7%) were previously *FLT3*^{pos}, and 2 (22.2%) gained a mutation. In the 6 patients who retained their *FLT3* mutation from first occurrence, 5 had *FLT3*-ITD AML and 1 had *FLT3*-TKD AML. In the 2 patients who gained *FLT3* mutations from first occurrence, 1 had *FLT3*-ITD AML and 1 had *FLT3*-TKD AML. Of 20 patients with *FLT3*^{neg} AML, 5 had missing *FLT3* testing data, 11 (55.0%) remained negative, and 4 (20.0%) lost their mutation (all patients with *FLT3*-ITD mutations). At first and second occurrence, a small subset of patients gained or lost other types of *FLT3* mutations.

Table 2 Method of *FLT3* mutation testing (FAS)

n (%)	*FLT3*^{pos}	*FLT3*^{neg}	Unknown/missing	Total
Genetic status available at baseline, *N*	172	472	6	650
PCR	121 (70.3)	353 (74.8)	0	474 (72.9)
NGS	37 (21.5)	88 (18.6)	0	125 (19.2)
Other	12 (7.0)	27 (5.7)	0	39 (6.0)
Missing	2 (1.2)	4 (0.8)	6 (100.0)	12 (1.8)
Genetic status available at 1st occurrence, *N*	45	121	125[a]	291[a]
PCR	33 (73.3)	87 (71.9)	1 (0.8)	121 (41.6)
NGS	4 (8.9)	9 (7.4)	0	13 (4.5)
Other	3 (6.7)	5 (4.1)	3 (2.4)	11 (3.8)
Missing	5 (11.1)	20 (16.5)	120 (96.0)	145 (49.8)

[a] *FLT3* assessment status was not available for 1 patient

EAS full analysis set, *FLT3 FMS*-like tyrosine kinase 3, *neg* negative, *NGS* next-generation sequencing, *PCR* polymerase chain reaction, *pos* positive



Baseline	1st occurrence		
	FLT3^{pos}	*FLT3*^{neg}	Total
FLT3^{pos}	37	18	55
FLT3^{neg}	7	103	110
Total	44	121	165

1st Occurrence	2nd occurrence		
	FLT3^{pos}	*FLT3*^{neg}	Total
FLT3^{pos}	6	4	10
FLT3^{neg}	2	11	13
Total	8	15	23

2nd Occurrence	3rd occurrence		
	FLT3^{pos}	*FLT3*^{neg}	Total
FLT3^{pos}	0	0	0
FLT3^{neg}	0	2	2
Total	0	2	2

Fig. 2 *FLT3* mutation gain and loss in consecutive relapse or refractory occurrences (FAS). *EAS* full analysis set, *FLT3 FMS*-like tyrosine kinase 3, *neg* negative, *pos* positive

Multivariate Logistic Regression Analyses

In a post hoc multivariate logistic regression analysis, baseline demographic and clinical characteristics such as age, sex, Eastern Cooperative Oncology Group performance status, ELN risk, other genetic mutations, and AML treatment showed no statistical significance with either *FLT3* mutation gain or loss (Supplementary Fig. 2).

FLT3 Mutation Characteristics and Other Genetic Mutations Associated with AML

In patients with *FLT3*-ITD mutations at baseline, the mean (standard deviation) allelic ratio was 0.6 (0.9), and the mean mutation length was 67.5 (84.8) base pairs (Supplementary Table 4). At subsequent occurrences, the mean allelic ratio decreased while the *FLT3*-ITD mutation length was variable.

At baseline, 423/650 (65.1%) patients had other genetic mutations associated with AML, of which nucleophosmin 1 (*NPM1;* 186/639 [29.1%]) and isocitrate dehydrogenase 1/2 (*IDH1/2;* 100/639 [15.6%]) were the most common. *NPM1* and *IDH1/2* mutations remained the most common in subsequent occurrences (Supplementary Table 5).

Overall, Disease-Free, and Event-Free Survival

Among all patients with a baseline visit ($n=649$), the median overall survival (OS) from baseline was not estimable (NE) in patients with *FLT3*^{pos} AML ($n=171$) and 20.3 (15.2–23.7) months in patients with *FLT3*^{neg} AML ($n=472$) (hazard ratio [HR] [95% CI] 0.6 [0.5–0.8). The median disease-free survival (DFS) from baseline was NE in both patients with *FLT3*^{pos} ($n=137$) and *FLT3*^{neg} ($n=297$) AML (HR [95% CI] 0.8 [0.6–1.1). The median event-free survival (EFS) from baseline was 17.2 (11.0–NE) months for *FLT3*^{pos} ($n=142$) and 10.4 (8.4–13.2) months for patients with *FLT3*^{neg} ($n=317$) AML (HR [95% CI] 0.8 [0.6–1.0]). The survival results of all patients with a first occurrence are presented in Table 3. OS, DFS, and EFS results by treatment subgroups are presented in Supplementary Table 6.

Composite Complete Remission

At baseline, CRc was achieved by 396/650 (60.9%) patients. A larger proportion of patients with *FLT3*^{pos} AML (125/172 [72.7%]) achieved CRc compared with patients with *FLT3*^{neg} AML (269/472 [57.0%]). CR was achieved by 350/650 (53.8%) patients (*FLT3*^{pos}, 109/172 [63.4%]; *FLT3*^{neg}, 239/472 [50.6%]). CRc rates at first occurrence are described in Supplementary Table 7. Data on patients who achieved CRc in different treatment subgroups are presented in Supplementary Table 8.

DISCUSSION

A deeper understanding of *FLT3* clonal evolution and its clinical consequences in AML is needed to optimize treatment for patients with *FLT3*^{pos} AML. This prospective, noninterventional study demonstrated that patients with AML undergo substantial clonal evolution over their disease course and are not tested in a timely manner.

In this study, >10% of patients gained or lost *FLT3* mutations at first occurrence of relapsed/refractory (R/R) AML. In line with these results, previous studies in predominantly FLT3 inhibitor-naive patient populations have reported clonal evolution rates of 6–22% [14, 18–20]. Interestingly, it has been previously reported that *FLT3* mutations were acquired 6 times more often than lost [19]; however, here, the rate of gain or loss of *FLT3* mutations was broadly similar, potentially associated with higher exposure to FLT3 inhibitors in this patient population as evidenced by the rates of patients receiving high-intensity chemotherapy with FLT3 inhibitors. In patients with *FLT3*^{neg} AML, gaining *FLT3*^{pos} AML clones is a common mechanism of resistance to chemotherapy and targeted therapies [21]. In contrast, in patients with *FLT3*^{pos} AML, *FLT3* mutation loss and gain of off-target mutations may reflect a compensatory mechanism for leukemic cell survival and proliferation

Table 3 OS, DFS, and EFS at baseline and first occurrence (FAS)

	FLT3^{pos}	*FLT3*^{neg}	Total
Baseline			
OS			
Baseline patients, *N*	171	472	649
Deaths, *n* (%)	66 (38.6)	255 (54.0)	327 (50.4)
Censored, *n* (%)	105 (61.4)	217 (46.0)	322 (49.6)
Median (95% CI), months[a]	NE (NE–NE)	20.3 (15.2–23.7)	22.8 (19.6–NE)
Hazard ratio (95% CI)[b]	0.6 (0.5–0.8)		
3-year OS rate (95% CI)	59.3 (51.2–66.5)	41.6 (36.4–46.7)	45.9 (41.5–50.1)
DFS			
Baseline patients who achieved CRc, *N*	137	297	436
Relapses or deaths, *n* (%)	51 (37.2)	137 (46.1)	190 (43.6)
Censored, *n* (%)	86 (62.8)	160 (53.9)	246 (56.4)
Median (95% CI), months[a]	NE (NE–NE)	NE (18.0–NE)	NE (26.3–NE)
Hazard ratio (95% CI)[b]	0.8 (0.6–1.1)		
3-year DFS rate (95% CI)	59.7 (50.2–68.0)	51.5 (45.3–57.3)	53.7 (48.6–58.6)
EFS			
Baseline patients in EFS analysis, *N*	142	317	461
Events, *n* (%)	75 (52.8)	194 (61.2)	271 (58.8)
Censored, *n* (%)	67 (47.2)	123 (38.8)	190 (41.2)
Median (95% CI), months[a]	17.2 (11.0–NE)	10.4 (8.4–13.2)	11.8 (10.2–15.5)
Hazard ratio (95% CI)[b]	0.8 (0.6–1.0)		
3-year EFS rate	46.1 (37.6–54.1)	37.3 (31.8–42.9)	39.9 (35.3–44.5)
1st occurrence			
OS			
Patients with first occurrence, *N*	45	121	291
Deaths, *n* (%)	26 (57.8)	73 (60.3)	201 (69.1)
Censored, *n* (%)	19 (42.2)	48 (39.7)	90 (30.9)
Median (95% CI), months[a]	12.2 (5.9–NE)	9.0 (5.8–12.8)	5.9 (5.0–7.8)
Hazard ratio (95% CI)[b]	0.8 (0.5–1.3)		
DFS			
Patients who achieved CRc after their first occurrence, *N*	19	46	94

Table 3 continued

	FLT3^{pos}	*FLT3*^{neg}	Total
Relapses or deaths, *n* (%)	8 (42.1)	17 (37.0)	39 (41.5)
Censored, *n* (%)	11 (57.9)	29 (63.0)	55 (58.5)
Median (95% CI), months[a]	NE (6.4–NE)	NE (8.7–NE)	NE (10.7–NE)
Hazard ratio (95% CI)[b]	1.1 (0.4–2.5)		
EFS			
First-occurrence patients in EFS analysis, *N*	20	60	131
Events, *n* (%)	11 (55.0)	32 (53.3)	84 (64.1)
Censored, *n* (%)	9 (45.0)	28 (46.7)	47 (35.9)
Median (95% CI), months[a]	13.6 (5.9–NE)	12.1 (8.3–NE)	9.6 (7.8–12.9)
Hazard ratio (95% CI)[b]	0.9 (0.5–1.8)		

[a]Based on Kaplan–Meier estimate

[b]Based on Cox proportional hazards model with *FLT3* mutation status as the only explanatory variable. Assuming proportional hazards, a hazard ratio > 1 indicates an increase in hazard rate for *FLT3* positive status

DFS disease-free survival, *CI* confidence interval, *CRc* composite complete remission, *EFS* event-free survival, *FAS* full analysis set, *FLT3 FMS*-like tyrosine kinase 3, *NE* not estimable, *neg* negative, *OS* overall survival, *pos* positive

when adapting to FLT3 inhibition [22]. An analysis of the RATIFY and AMLSG 16–10 trials revealed that 46% of patients treated with midostaurin lost their *FLT3*-ITD mutation at R/R disease versus 19% of patients not treated with midostaurin [22]. Moreover, patients who lost their *FLT3*-ITD mutation acquired mutations in other signaling pathways [22]. A clearer understanding of mutational factors involved in *FLT3* clonal evolution and their relationship with FLT3 inhibitors is needed.

Despite the availability of several FLT3 inhibitors and guideline recommendations for timely testing [12], a substantial number of patients (40%) had an unknown *FLT3* testing status at first or second occurrence of R/R disease, suggesting a decrease in testing rates. These results were similar to real-world testing rates of 39–47% [14, 20] in patients with R/R AML. In a small number of patients with *FLT3*-ITD data, the allelic ratio tended to decrease during progression, and the mutation length was variable. *FLT3*-ITD mutation length does not appear to have any prognostic impact [23]; however, treatment with FLT3 inhibitors is especially effective in patients with a high allelic ratio [24].

In addition to *FLT3* mutations, other genetic mutations were observed in over half of the patient population at baseline, with a decrease in testing rates at subsequent occurrences. *NPM1* and *IDH1/2* mutations were the most common at baseline and subsequent occurrences. These results were in line with what is known about AML, as *NPM1* is the most commonly mutated gene in adult AML, and *IDH1/2* mutations are associated with *NPM1* mutations [25], while *FLT3* and *NPM1* are often co-mutated [26]. The presence of *NPM1* and *IDH1/2* mutations can help guide treatment decisions as several treatment strategies have been effective in the treatment of patients with *NPM1* mutations [27], with others such as menin inhibitors being investigated [28]. Likewise, IDH inhibitors have demonstrated efficacy in the treatment of patients with R/R AML [29], further highlighting the need for additional genetic testing.

Compared with the established literature [11, 30], here patients with *FLT3*^{pos} AML had better

OS results compared with patients with *FLT3*^{neg} AML from baseline but not from first occurrence. This was likely owing to the availability of FLT3 inhibitors for the treatment of patients with *FLT3*^{pos} AML compared with the lack of personalized options for patients with *FLT3*^{neg} AML and general treatment heterogeneity between patients. In addition, patients with *FLT3*^{pos} AML were younger compared with patients with *FLT3*^{neg} AML, and consequently, likely fitter for a more aggressive standard high-intensity chemotherapy-based approach. In patients receiving high-intensity chemotherapy at baseline, a similar trend was observed between patients with *FLT3*^{pos} and *FLT3*^{neg} AML. These results are in line with the updated ELN 2022 recommendations, which now categorize patients with *FLT3*-ITD AML with or without *NPM1* co-mutations in the intermediate-risk instead of the high-risk group, on the basis of methodological issues with standardizing the assay to measure the *FLT3*-ITD allelic ratio, the modifying impact of midostaurin-based therapy on newly diagnosed patients with *FLT3*-ITD AML, and the increasing role of measurable residual disease (MRD) in treatment decisions [12].

This study had several limitations. As this was a descriptive study, it was not statistically powered to compare outcomes between patients with *FLT3*^{pos} and *FLT3*^{neg} AML. Interpretation of clonal evolution and clinical outcomes from later disease stages or different treatment subgroups may be limited by small sample sizes, while certain analyses such as comparisons between patients with *FLT3*-ITD AML and *FLT3*-TKD AML were also not possible owing to the small sample sizes of the different mutation groups, especially at first occurrence and beyond. Furthermore, only a small proportion of patients had complete follow-up, owing to early termination of the study. Survival results may have been biased owing to the early termination of the study, which resulted in the censoring of 44.0% of patients. In addition, as guidelines began to recommend additional molecular re-evaluation at relapse in 2022 [12], patients enrolled before 2022 were possibly missing genetic data more frequently and missing information on other *FLT3* mutations apart from *FLT3*-ITD and *FLT3*-TKD. As genetic testing was performed per the local standard of care, the extent of testing, assessment methodologies, and limits of detection varied between study sites and countries, and MRD data were not collected. Overall, PCR was the most commonly used testing method; however, particularly at first or second occurrence, smaller *FLT3* clones may have gone undetected owing to the lower sensitivity from the higher limit of detection associated with PCR techniques [31–33]. Prior research has shown that *FLT3*-ITD MRD can occur at levels below the threshold of conventional PCR with capillary electrophoresis, while NGS techniques can identify these low-level clones with greater sensitivity [34]. When the study started in 2020, PCR-based testing was the standard of care; however, currently, there is not only a strong shift toward using NGS-based methods for *FLT3* assessment, at relapse or refractory disease, but also pre- and post-hematopoietic stem cell transplantation to better detect and characterize MRD [35]. Moreover, NGS-based methods are now recommended for the ongoing management of AML as per National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN Guidelines) [36]. This evolution in testing methods enables the detection of *FLT3*-ITD expression in patients with previously undetectable levels and subsequently would allow for a more targeted use of FLT3 inhibitors to improve clinical outcomes. It is worth noting that even though PCR was the most commonly used testing method, a notable proportion of patients experienced *FLT3* clonal evolution. Given the increased sensitivity of NGS, it is possible that fewer apparent losses of *FLT3* mutations would have been observed had NGS been used throughout. Future studies should use NGS to more comprehensively assess and track clonal evolution.

CONCLUSIONS

This study demonstrated that the *FLT3* mutation status of patients with AML undergoes dynamic changes throughout disease course, with a

notable proportion of patients experiencing a gain or loss of *FLT3* mutations. These results emphasize the need for timely and appropriate *FLT3* mutation retesting throughout the disease course, as increased testing with appropriate sensitivity thresholds may aid in further personalizing treatment options.

ACKNOWLEDGMENTS

We thank the participants of the study.

Medical Writing/Editorial Assistance. The authors thank Pedro de Campos Silva, PhD, CMPP, of Lumanity Scientific Inc., for providing medical writing support, which was funded by Astellas Pharma Inc. in accordance with Good Publication Practice (GPP 2022) guidelines.

Author Contributions. Conceptualization: Cristina Papayannidis, Irina Amitai, Pascal Turlure, Ann De Becker, Felix Mensah, Dina Elsouda, Paresh Vyas, Blanca Boluda, Ioanna Vardounioti, and Jose Palacios-Fabrega. Formal analysis and investigation: Cristina Papayannidis, Irina Amitai, Pascal Turlure, Ann De Becker, Felix Mensah, Dina Elsouda, Paresh Vyas, Blanca Boluda, Ioanna Vardounioti, and Jose Palacios-Fabrega. Data acquisition: Cristina Papayannidis, Irina Amitai, Pascal Turlure, Ann De Becker, Felix Mensah, Dina Elsouda, Paresh Vyas, Blanca Boluda, Ioanna Vardounioti, and Jose Palacios-Fabrega. Writing—original draft preparation: all authors. Writing—review and editing: all authors.

Funding. This study and its resultant publications were initiated and funded by Astellas Pharma Inc. Medical writing support was provided by Pedro de Campos Silva, PhD, of Lumanity Scientific Inc., funded by Astellas Pharma Inc. The journal's Rapid Service Fee was funded by Astellas Pharma Inc.

Data Availability. Details for how researchers may request access to anonymized participant level data, trial level data, and protocols from Astellas-sponsored clinical trials can be found at https://www.clinicaltrials.astellas.com/transparency/.

Declarations

Conflict of Interest. Cristina Papayannidis received payment or honoraria fees from AbbVie, Amgen, Astellas, Bristol Myers Squibb, Istituto Gentili, Incyte, Johnson & Johnson, Menarini/Stemline, Novartis, Pfizer, and Servier; and served on a data safety monitoring board or advisory board for AbbVie, Astellas, Blueprint Medicines, Delbert Pharma, GSK, Istituto Gentili, Janssen, Jazz Pharmaceuticals, Novartis, Pfizer, and Syndax. Irina Amitai received payment or honoraria from AbbVie and Novartis; received congress attendance support from Takeda and Roche; and served on a data safety monitoring board or advisory board for Astellas, Menarini/Stemline, and Neopharm Israel. Pascal Turlure has no conflicts of interest to declare. Ann De Becker served on a data safety monitoring board or advisory board for Novartis, Bristol Myers Squibb, and Takeda; and received support for attending congresses for Pfizer, Bristol Myers Squibb, and Takeda. Felix Mensah received payment or honoraria fees from Eli Lilly and Sobi. Dina Elsouda, Ioanna Vardounioti, and Jose Alejandro Palacios-Fabrega are employees of Astellas. Paresh Vyas and Blanca Boluda have no conflicts of interest to declare.

Ethical Approval. This study adhered to International Council for Harmonisation guidelines for Good Clinical Practice, the Declaration of Helsinki, and applicable regulations. Written informed consent was obtained from all patients. The protocol and relevant documents were approved by the IRBs/IECs at each study site. Data were analyzed by the sponsor, with all authors having access to the study data.

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