ORIGINAL RESEARCH

The impact of FLT3 mutation clearance and treatment response after gilteritinib therapy on overall survival in patients with FLT3 mutation–positive relapsed/refractory acute myeloid leukemia

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Abstract

The FLT3 inhibitor gilteritinib has clinical activity in patients with FLT3-mutated (FLT3mut+) relapsed/refractory (R/R) acute myeloid leukemia (AML). The impact of FLT3 mutation clearance and the achievement of composite complete remission (CRc) and complete remission/complete remission with partial hematologic recovery (CR/CRh) on overall survival (OS) in patients with FLT3mut+ R/R AML treated with single-agent gilteritinib in a phase 1/2 trial were evaluated. Using next-generation sequencing, a FLT3-ITD variant allele frequency of ≤10−4 was used to define FLT3-ITD clearance in patients with no morphologic leukemia (ie, CRc). A total of 108 patients with FLT3-ITD-positive (FLT3-ITD+) R/R AML were analyzed; 95 of these patients had received ≥80-mg/day gilteritinib. Ten of the 95 patients had FLT3-ITD clearance; eight of these 10 patients achieved CRc and were considered negative for measurable residual disease. There was a trend toward longer OS in patients who attained CRc with FLT3-ITD clearance (131.4 weeks) versus those who achieved CRc and did not have FLT3-ITD clearance (n = 41; 43.3 weeks; HR = 0.416; p = 0.066).

Among patients treated with ≥80-mg/day gilteritinib who achieved CR/CRh (n = 24), seven had FLT3-ITD clearance. Among patients who achieved CR/CRh, those who achieved CR/CRh had a longer median OS (70.6 weeks) and higher 52-week survival probability (66.7%) than patients who did not achieve CR/CRh (n = 71; median OS, 41.7 weeks; 52-week survival probability, 20.2%). Overall, these data suggest that gilteritinib can induce deep molecular responses in patients with FLT3-ITD+ R/R AML, and in the setting of CRc or CR/CRh, these responses may be associated with prolonged survival.

KEYWORDS

FLT3 inhibitor, Fms-like tyrosine kinase 3, internal tandem duplication, morphologic remission
INTRODUCTION

Measurable residual disease (MRD) in AML refers to the persistence of residual leukemic cells below clinically detectable levels despite achievement of morphologic remission.\(^1\) The presence of MRD after achievement of morphologic complete remission (CR) has emerged as an independent prognostic marker of increased relapse risk and shortened survival in patients with AML.\(^1,2\)

Common techniques to detect MRD include multi-parameter flow cytometry (MFC) and reverse transcription quantitative polymerase chain reaction (RT-qPCR). Despite the wide applicability of MFC, the sensitivity of detection of leukemia-associated immunophenotypic cells is only 0.1% to 0.01% (10\(^{-3}\) to 10\(^{-4}\)) and requires comparison against diagnostic specimens, which are not always available.\(^3\) Although use of optimized RT-qPCR to detect MRD by amplifying leukemia-associated genetic aberrations has increased sensitivity to a 10\(^{-4}\) to 10\(^{-6}\) range, these assays depend on the presence of specific mutations (\(NPM1\) and \(core-binding factor\)) and require standard curves for reference.\(^4\) Digital PCR improves on RT-qPCR sensitivity without the need for a standard curve.\(^5\) Next-generation sequencing (NGS) has emerged as a valuable tool for monitoring MRD in AML because it enables comprehensive and simultaneous detection of patient-specific somatic mutations indicating subclinical disease that is undetected by RT-qPCR, has a sensitivity of 10\(^{-3}\) to 10\(^{-6}\), and is applicable in the majority of AML cases.\(^2,4,5\)

In AML, the likelihood of MRD after chemotherapy is substantially greater in patients harboring activating \(FLT3\) internal tandem duplication (\(FLT3\)-ITD) mutations than in patients with wild-type \(FLT3\).\(^6\) However, as \(FLT3\)-ITD mutations do not always persist at relapse, systematic monitoring of \(FLT3\)-ITD mutations in patients in first remission remains controversial.\(^7\) With the emergence of approved \(FLT3\) inhibitors for AML, accurate assessment of MRD will establish the long-term efficacy of these agents. Conventional PCR assays using genomic DNA for \(FLT3\) mutation detection are confounded by template bias from the wild-type allele,\(^8\) resulting in low sensitivity (~1%). This challenge can be overcome through a combined PCR-NGS approach, with bioinformatics designed to detect \(FLT3\)-ITD insertions of normal coding sequence. This approach enables identification of clonal composition and the dominance of \(FLT3\)-ITD mutations.\(^9,10\) Although \(FLT3\)-ITD–positive (+) clones are unstable, appearing or disappearing at relapse,\(^11\) considerable inter-individual heterogeneity in ITD length\(^12,13\) facilitates detection specificity.\(^9\) A clinical response to a \(FLT3\) inhibitor without a reduction in \(FLT3\) allele burden may reflect differentiation of the leukemic clone.\(^14,15\)

**Gilteritinib** is an oral \(FLT3\) inhibitor with activity against \(FLT3\)-ITD and \(FLT3\) tyrosine kinase domain (TKD) mutations.\(^16,17\) In the phase 1/2 CHRYSLIS study, gilteritinib was well tolerated and induced antileukemic activity at doses ≥80 mg/day in patients with relapsed/refractory (R/R) \(FLT3\)-mutated (\(FLT3^{\text{mut+}}\)) AML.\(^18\) The phase 3 ADMIRAL trial demonstrated the superior efficacy of 120-mg/day gilteritinib compared with salvage chemotherapy in patients with R/R \(FLT3^{\text{mut+}}\) AML.\(^19,20\)

A sensitive and specific NGS-based assay used to detect MRD in a \(FLT3\)-ITD+ R/R AML patient subset who achieved morphologic CR with gilteritinib in the CHRYSLIS study demonstrated a relationship between \(FLT3\)-ITD mutation burden and overall survival (OS).\(^9\)

\(FLT3\) inhibitors can induce differentiation and cytotoxicity in bone marrow blasts, resulting in CR with incomplete hematologic recovery (CRi).\(^18,21\) Studies of \(FLT3\) inhibitors in R/R AML report the composite CR (CRc) rate, which is the sum of CR, CRi, and CR with incomplete platelet recovery (CRp).\(^18,21\) A CRh response describes bone marrow blast clearance with partial, clinically significant, hematologic recovery that is not defined by other response criteria.\(^22\) The impact of CRc or CR/CRh on \(FLT3\)-ITD clearance and long-term survival in patients with \(FLT3^{\text{mut+}}\) R/R AML has not been evaluated.

A previous analysis of a \(FLT3\)-ITD+ R/R AML patient subgroup from the CHRYSLIS study who achieved CRc with 120-mg or 200-mg gilteritinib demonstrated longer survival in MRD-negative (MRD−) patients than in MRD-positive (MRD+) patients over a 1- to 2-year period.\(^9\) We evaluated the impact of \(FLT3\)-ITD mutation clearance and achievement of CRc or CR/CRh on survival beyond 3 years in a larger \(FLT3\)-ITD+ R/R AML patient subset who received 20- to 450-mg/day gilteritinib in the CHRYSLIS study.\(^18\)

METHODS

2.1 | CHRYSLIS study design and patient population

CHRYSLIS was a phase 1/2, open-label, dose-escalation/dose-expansion study of once-daily oral gilteritinib (20–450 mg) in patients with R/R AML.\(^18\) Adult patients (aged ≥18 years) with primary or secondary AML who were either refractory to ≥1 cycle of induction chemotherapy, or had relapsed after achieving remission with a prior therapy, were enrolled.\(^18\) Patients with \(FLT3\)-ITD mutations or \(FLT3\)-TKD point mutations were enrolled into each expanded dose level, including the dose-escalation cohorts (20-, 40-, 80-, 120-, 200-, 300-, and 450-mg/day gilteritinib).\(^18\) The decision to escalate to the next dose level was based on the
assessments of grade 2 adverse events and dose-limiting toxicities.\(^{18}\)

### 2.2 | Assessment of mutation clearance

The presence of FLT3-ITD mutations was assessed in bone marrow aspirates from patients with FLT3-ITD+ R/R AML who had received 20- to 450-mg/day gilteritinib and had samples available at baseline and \(≥1\) additional post-baseline time point prior to hematopoietic stem cell transplantation (HSCT). The FLT3-ITD mutation assay was performed according to previously published methods.\(^{9}\) Specifically, using genomic DNA, FLT3 exons 14 and 15 were amplified by PCR and FLT3-ITD and total FLT3 alleles were subsequently quantified by NGS using an Illumina® MiSeq platform.\(^{9}\) Read depths of \(≥100,000\) reads per sample were implemented, and operating characteristics were linear to \(10^{-4}\) for the range of ITD lengths using cell lines spiked to normal blood or bone marrow.\(^{9}\) Data were analyzed using proprietary software. FLT3-ITD variant allele frequency (VAF) was defined as the FLT3-ITD to total FLT3 frequency; FLT3-ITD clearance was defined as a FLT3-ITD VAF of \(≤10^{-4}\). A patient was classified as having FLT3-ITD clearance if they had a FLT3-ITD VAF of \(≤10^{-4}\) at any post-baseline time point prior to HSCT. A Cox regression model, with Kaplan–Meier estimation, was used to evaluate the impact of FLT3 VAF on OS.

### 2.3 | Assessment of the relationship between treatment response, mutation clearance, and OS

The impact of CRc or CR/CRh with FLT3-ITD mutation clearance on OS was assessed in a subgroup of patients with FLT3-ITD+ R/R AML who had received \(≥80\)-mg/day gilteritinib, which induced maximum inhibition of FLT3 receptor auto-phosphorylation and antileukemic response.\(^{18}\) The impact of achieving CR/CRh on OS was assessed in a subgroup of patients with FLT3-ITD+ R/R AML who had received 120 mg/day, which was established as the recommended dose in the phase 1/2 CHRYSALES study,\(^{18}\) and was evaluated against salvage chemotherapy in the phase 3 ADMIRAL trial.\(^{20}\) Composite complete remission was defined as CR plus CRp plus CRi. Patients who achieved CR achieved morphologic leukemia-free status, had an absolute neutrophil count (ANC) of \(>1 \times 10^9/L\) and an absolute platelet count of \(≥100 \times 10^9/L\), normal bone marrow differential with \(<5\%\) bone marrow blasts, red blood cell and platelet transfusion independence, and no evidence of extramedullary leukemia. Patients who achieved CRp met all of the criteria for CR but had a platelet count of \(<100 \times 10^9/L\) in the absence of transfusions. Patients who achieved CRi met all of the criteria for CR but had residual neutropenia (ANC \(< 1 \times 10^9/L\)) with or without platelet recovery. Patients who achieved CRh had bone marrow blasts \(<5\%\), ANC \(≥ 0.5 \times 10^9/L\), absolute platelet count of \(≥50 \times 10^9/L\), and no evidence of extramedullary leukemia.

### 2.4 | Statement of ethics

The CHRYSALES study was conducted in accordance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice guidelines, the principles of informed consent, and the requirements of public registration of clinical trials. Approval was obtained from site-specific institutional review boards. Written informed consent was obtained from each patient at the time of enrollment.

### 3 | RESULTS

#### 3.1 | Analysis population

A total of 108 patients out of 178 with FLT3-ITD+ R/R AML in the CHRYSALES study were analyzed for FLT3-ITD mutation clearance. Of these patients, 40 (37%) had samples available for analysis at one or two post-baseline time points (treatment cycles 3 and 4) and 21 (19.4%) had samples available for analysis at \(≥2\) post-baseline time points (up to treatment cycle 28). Patients with evidence of FLT3-ITD mutation clearance only after HSCT were classified as not having FLT3-ITD clearance.

Demographic and baseline characteristics of patients assessed for mutation clearance were representative of the entire CHRYSALES R/R AML population (Table 1).\(^{18}\) Overall, 95 of 108 patients (88%) had received \(≥80\)-mg/day gilteritinib. NPM1 mutations (based on local laboratory testing) were also present in 37 of 108 patients (34.3%)—33 of whom had received \(≥80\)-mg/day gilteritinib. A total of 10 of the 108 patients had achieved FLT3-ITD mutation clearance with gilteritinib therapy; all 10 of these patients had received \(≥80\)-mg doses of gilteritinib. Median FLT3-ITD VAF at baseline was similar in patients with FLT3-ITD mutation clearance and in those without FLT3-ITD mutation clearance; median ITD length was 48 bp (range, 3–204). Among all patients receiving gilteritinib (\(n = 108\)), CR rates with prior AML therapy were higher among patients with FLT3-ITD mutation clearance (80%; \(n = 8/10\)) than in those without FLT3-ITD mutation clearance (58%; \(n = 57/98\)), and the median duration of CR achieved with prior AML therapy was also longer in patients with FLT3-ITD mutation clearance (7.9 vs. 4.1 months, respectively).
| Characteristics                              | Gilteritinib ≥ 80 mg/day subgroup | Total analysis population |
|---------------------------------------------|----------------------------------|---------------------------|
|                                             | FLT3-ITD Cleared (n = 10)        | FLT3-ITD Not Cleared (n = 85) | Combined (N = 95) | FLT3-ITD Cleared (n = 10) | FLT3-ITD Not Cleared (n = 98) | Combined (N = 108) |
| Median age, years (range)                   | 59.5 (29–76)                    | 61.0 (21–86)               | 61.0 (21–86)      | 59.5 (29–76)               | 60.5 (21–86)               | 60.5 (21–86) |
| Median FLT3 variant allele frequency (range) | 0.4205 (0.041–0.827)            | 0.407 (0.036–0.986)        | 0.407 (0.036–0.986) | 0.4205 (0.041–0.827)        | 0.4056 (0.036–0.986)       | 0.4056 (0.036–0.986) |
| Sex, n (%)                                  |                                  |                           |                   |                           |                           |                           |
| Male                                        | 3 (30)                           | 42 (49)                    | 45 (48)           | 3 (30)                     | 49 (50)                     | 52 (48)                     |
| Female                                      | 7 (70)                           | 43 (51)                    | 50 (53)           | 8 (70)                     | 49 (50)                     | 56 (52)                     |
| AML type, n (%)                             |                                  |                           |                   |                           |                           |                           |
| De novo                                     | 9 (90)                           | 72 (85)                    | 81 (85)           | 9 (90)                     | 83 (85)                     | 92 (85)                     |
| Secondary                                   | 1 (10)                           | 13 (15)                    | 14 (15)           | 1 (10)                     | 15 (15)                     | 16 (15)                     |
| Cytogenetic risk status, n (%)              |                                  |                           |                   |                           |                           |                           |
| Favorable                                    | 1 (10)                           | 2 (2)                      | 3 (3)             | 1 (10)                     | 2 (2)                       | 3 (3)                       |
| Intermediate                                | 8 (80)                           | 56 (66)                    | 64 (67)           | 8 (80)                     | 65 (66)                     | 73 (68)                     |
| Unfavorable                                  | 0                                | 12 (14)                    | 12 (13)           | 0                          | 14 (14)                     | 14 (13)                     |
| Unknown/missing                              | 1 (10)                           | 15 (18)                    | 16 (17)           | 1 (10)                     | 17 (17)                     | 18 (17)                     |
| Lines of prior AML therapy, n (%)           |                                  |                           |                   |                           |                           |                           |
| 1                                           | 4 (40)                           | 29 (34)                    | 33 (35)           | 4 (40)                     | 32 (33)                     | 36 (33)                     |
| 2                                           | 3 (30)                           | 25 (29)                    | 28 (29)           | 3 (30)                     | 27 (28)                     | 30 (28)                     |
| ≥3                                          | 3 (30)                           | 31 (36)                    | 34 (36)           | 3 (30)                     | 39 (40)                     | 42 (39)                     |
| Prior transplantation, n (%)                |                                  |                           |                   |                           |                           |                           |
| Yes                                         | 5 (50)                           | 20 (24)                    | 25 (26)           | 5 (50)                     | 22 (22)                     | 27 (25)                     |
| No                                          | 5 (50)                           | 65 (76)                    | 70 (74)           | 5 (50)                     | 76 (78)                     | 81 (75)                     |
| Prior TKI therapy, n (%)                    |                                  |                           |                   |                           |                           |                           |
| Yes                                         | 3 (30)                           | 24 (28)                    | 27 (28)           | 3 (30)                     | 30 (31)                     | 33 (31)                     |
| No                                          | 7 (70)                           | 61 (72)                    | 68 (72)           | 7 (70)                     | 68 (69)                     | 75 (69)                     |
| Best response to prior AML therapy, n (%)   |                                  |                           |                   |                           |                           |                           |
| CR                                          | 8 (80)                           | 49 (58)                    | 57 (60)           | 8 (80)                     | 57 (58)                     | 65 (60)                     |
| PR                                          | 1 (10)                           | 16 (19)                    | 17 (18)           | 1 (10)                     | 17 (17)                     | 18 (17)                     |
| Duration of response to prior AML therapy   |                                  |                           |                   |                           |                           |                           |
| Median duration of CR, months (range)       | 7.9 (1.7–15.8)                  | 3.9 (0.8–64.1)             | 4.6 (0.8–64.1)    | 7.9 (1.7–15.8)             | 4.1 (0.8–64.1)             | 4.9 (0.8–64.1)             |
| Median duration of PR, months (range)       | 1.0 (1.0–8.9)                   | 1.3 (0.4–12.0)             | 1.0 (0.4–12.0)    | 1.0 (1.0–8.9)              | 1.7 (0.4–12.0)             | 1.3 (0.4–12.0)             |

Percentages are rounded to whole numbers.

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; ITD, internal tandem duplication; PR, partial remission; R/R, relapsed/refractory; TKI, tyrosine kinase inhibitor.
3.2 | Overall survival according to mutation clearance status

Among the 95 patients who received ≥80-mg/day gilteritinib, 10 had FLT3-ITD clearance at any post-baseline time point. Median OS was 76.8 weeks (95% CI: 18.6, not reached) in patients who had FLT3-ITD clearance and 30.6 weeks (95% CI: 22.4, 37.7) in those who did not have FLT3-ITD clearance (HR = 0.663; 95% CI: 0.298, 1.475).

3.3 | Impact of mutation clearance and achievement of CRc on overall survival

Of the 95 patients who received ≥80-mg/day gilteritinib, 49 (51.6%) had a best overall response of CRc (Table 2). Patients who achieved CRc and had FLT3-ITD clearance (n = 8) had a trend toward longer median OS (131.4 weeks; 95% CI: 18.6, not reached) than those who achieved CRc and did not have FLT3-ITD clearance (n = 41; median OS: 43.3 weeks; 95% CI: 27.7, 56.9; HR = 0.416 [95% CI: 0.159, 1.086]) and did not reach statistical significance (p = 0.066; Figure 1). Two of the eight patients (25.0%) who achieved CRc with FLT3-ITD clearance underwent HSCT after achieving CRc. Of the 41 patients who achieved CRc without FLT3-ITD clearance, 15 (36.6%) underwent HSCT after achieving CRc.

Overall, the median duration of CRc in patients who had FLT3-ITD clearance was 60.0 weeks (95% CI: 12.3, not reached) and 12.1 weeks (95% CI: 8.3–27.3) in those who did not have FLT3-ITD clearance (HR = 0.412; 95% CI: 0.139, 1.226; Figure 2). Among patients who had received ≥80-mg/day gilteritinib and had prior treatment with a FLT3 TKI (n = 27), two of three patients achieved CRc with FLT3-ITD clearance; 11 of 24 patients achieved CRc without FLT3-ITD clearance.

3.4 | Impact of FLT3 mutation clearance status and achievement of CR/CRh on OS

Overall, 24 of 95 patients (25.3%) who received ≥80-mg/day gilteritinib had a best overall response of CR/CRh; 71 patients did not achieve CR/CRh (Table 3). Of the 24 patients with CR/CRh, seven (29.2%) had FLT3-ITD clearance and 17 (70.8%) did not have FLT3-ITD clearance. Of the seven patients with FLT3-ITD clearance, five (71.4%) achieved CR and two (28.6%) achieved CRh. Of the 17 who did not have FLT3-ITD clearance, seven (41.2%) achieved CR and 10 (58.8%) achieved CRh. Three of the 71 patients who did not

### Table 2

| Mutation clearance status | CRc (n = 49) | No CRc (n = 46) | Total (N = 95) |
|---------------------------|-------------|----------------|---------------|
| FLT3-ITD cleared          | 8 (16.3%)   | 2 (4.3%)       | 10 (10.5%)    |
| FLT3-ITD not cleared      | 41 (83.7%)  | 44 (95.7%)     | 85 (89.5%)    |

Abbreviations: AML, acute myeloid leukemia; CRc, composite complete remission; ITD, internal tandem duplication; R/R, relapsed/refractory.

**Figure 1** Overall survival in patients with FLT3-ITD+ R/R AML who received ≥80-mg/day gilteritinib and had a best overall response of CRc stratified by FLT3 mutation clearance status. AML, acute myeloid leukemia; CI, confidence interval; CRc, composite complete remission; ITD, internal tandem duplication; and R/R, relapsed/refractory.
achieve CR/CRh (4.2%) had FLT3-ITD clearance. For patients who achieved CR/CRh and had FLT3-ITD clearance, the median duration of CR/CRh had not been reached; those who did not have FLT3-ITD clearance had a median duration of CR/CRh of 19.4 weeks (HR = 0.454; 95% CI: 0.117, 1.752; Figure 3).

Based on aggregate data pertaining to the toxicity and antileukemic activity of gilteritinib, a dose of 120 mg/day was selected as the starting dose of gilteritinib as single-agent therapy in subsequent clinical trials. Of the 56 FLT3-ITD+ patients in the 120-mg/day dose cohort, 34 were assessed for FLT3 mutation clearance, and six of the 34 patients (17.6%) had FLT3-ITD clearance. Nine of the 34 patients (26.5%) achieved a best overall response of CR/CRh. After excluding patients with an OS duration that was less than the median time to reach CR/CRh, patients who achieved CR/CRh had a longer median OS and greater survival probability than patients who did not achieve CR/CRh. The median OS in the CR/CRh and no CR/CRh cohorts was 70.6 weeks (95% CI: 27.1, not reached) and 41.7 weeks (95% CI: 30.4, 51.7), respectively (Figure S1). The survival probability at 52 weeks was 66.7% (95% CI: 33.7, 86.0) versus 20.2% (95% CI: 9.5, 33.6), respectively.

TABLE 3  FLT3 mutation clearance in patients with FLT3-ITD+ R/R AML according to CR/CRh response after treatment with ≥80-mg/day gilteritinib

| Mutation clearance status | CR/CRh (n = 24) | No CR/CRh (n = 71) | Total (N = 95) |
|---------------------------|-----------------|-------------------|---------------|
| FLT3-ITD cleared          | 7 (29.2%)       | 3 (4.2%)          | 10 (10.5%)    |
| FLT3-ITD not cleared      | 17 (70.8%)      | 68 (95.8%)        | 85 (89.5%)    |

Abbreviations: AML, acute myeloid leukemia; CR, complete remission; CRh, complete remission with partial hematologic recovery; ITD, internal tandem duplication; R/R, relapsed/refractory.

Expanding knowledge of the genomic landscape in AML, as well as the recent availability of multiple targeted therapies for patients with newly diagnosed or R/R AML, has substantially reshaped the AML treatment paradigm. However, these advances are accompanied by questions and challenges regarding assessment of treatment efficacy over time and identification of the most accurate and clinically meaningful parameters of long-term response. As a post-treatment biomarker, mutation clearance has the potential to further define the quality of response to a given therapy, which can augment and refine prognosis in AML.23 To this end, European LeukemiaNet (ELN) has recently endorsed the achievement of MRD − CR as a key treatment goal in AML using either flow cytometry or real-time PCR approaches, as appropriate.7,24

In the current analysis, we evaluated treatment response and mutation clearance using a combined PCR- and NGS-based approach in a subgroup of patients with FLT3+ R/R AML who received gilteritinib therapy. Our findings showed that gilteritinib induced deep molecular responses and FLT3-ITD clearance, as defined by a low FLT3-ITD allele burden, in heavily pretreated patients with FLT3-ITD+ R/R AML. Results from this analysis demonstrated a potential relationship between achievement of FLT3-ITD clearance and longer
OS in these patients. Patients who achieved CRc or CR/CRh with gilteritinib therapy had a higher rate of FLT3-ITD clearance and longer OS than those who did not achieve CRc or CR/CRh.

A similar relationship between mutation clearance and treatment response has been observed in studies of isocitrate dehydrogenase (IDH) inhibitors in patients with IDH1- or IDH2-mutated R/R AML.25,26 In patients treated with the IDH1 inhibitor ivosidenib, the clearance of IDH1 mutations was associated with longer OS and remission duration. Median OS was 14.5 months in patients with IDH1 mutation clearance and 10.2 months in patients without IDH1 mutation clearance; median duration of CR/CRh was 11.1 and 6.5 months, respectively.25 Following treatment with the IDH2 inhibitor enasidenib, 12 patients with IDH2-mutated R/R AML had IDH2-R140 mutation clearance and 10 of these patients achieved morphologic CR.26 Median OS was longer among patients who achieved morphologic CR with mutation clearance (22.9 months) than in those who did not achieve morphologic CR (8.8 months; p = 0.0153). However, among the 35 patients who achieved morphologic CR, there was no significant difference in OS between patients who had mutation clearance (n = 10; median OS, 22.9 months) and those who did not have mutation clearance (n = 25; median OS, 20.7 months).26

In regard to treatment response, the goal of induction therapy is to achieve morphologic CR; however, R/R patients receiving gilteritinib or other FLT3 inhibitors frequently achieve less well-validated responses, such as CRi.18,21 It remains to be seen whether CRi achieved after FLT3-targeted therapy reshapes this perception. The use of CRh as a marker of response was implemented in the pivotal phase 3 ADMIRAL trial, comparing gilteritinib with salvage chemotherapy in FLT3mut+ R/R AML,20 and has also been implemented in the phase 1 trial of the IDH1 inhibitor, ivosidenib, in IDH1-mutated R/R AML.25 Given the dearth of evidence regarding the impact of CRi, CRp, or CRh on mutation clearance after FLT3 inhibitor therapy in patients with FLT3-ITD+ AML, further evaluation of these responses is clearly warranted.

It is difficult to identify the specific variables that are likely to yield the best outcomes in patients treated with gilteritinib—whether a bias toward HSCT exists in patients who had FLT3-ITD clearance, whether patients had FLT3-ITD clearance before HSCT, or whether patients who had FLT3-ITD clearance received gilteritinib maintenance therapy. However, our small sample size restricts extrapolation of our findings to the larger R/R AML population. We did not evaluate the impact of commonly occurring co-mutations such as NPM1, which could potentially influence treatment response and OS. We also did not perform MFC as a means of cross-platform validation of MRD– status in patients who achieved CR or CRc and had FLT3-ITD clearance. Because results from our survival analyses did not reach statistical significance, the validity of these observations should be further tested in an appropriately powered independent validation cohort. We observed that most patients with FLT3-ITD mutation clearance following gilteritinib therapy had also achieved CR with prior AML therapy and had a longer duration of response to prior treatment than patients without FLT3-ITD mutation clearance. However, due to the small

![FIGURE 3](image-url) Duration of CR/CRh according to FLT3 mutation clearance status in patients with FLT3-ITD+ R/R AML who received ≥80-mg/day gilteritinib. AML, acute myeloid leukemia; CI, confidence interval; CR, complete remission; CRh, complete remission with partial hematologic recovery; ITD, internal tandem duplication; and R/R, relapsed/refractory
sample size, these observations should be interpreted with caution and further investigated in a larger population.

It is important to note that FLT3-ITD mutations occur as late hits in leukemogenesis and often present in subclones. Thus, clearance of FLT3-ITD mutations is not necessarily proportional to reduction in the percentage of leukemic blasts or the clearance of clonal/pre-leukemic cells. Recent evidence demonstrates that although gilteritinib therapy can clear FLT3-ITD mutations, clones may persist in patients on the basis of karyotype or co-mutations, suggesting that clearance of a subclone may not be indicative of MRD—status according to conventional standards. Clearance of all mutations in patients with FLT3 mut+ R/R AML who received gilteritinib therapy appears to be limited to patients who relapsed after prior HSCT. We did not assess patients with baseline point mutations in the FLT3 tyrosine kinase domain due to the low number of patients harboring these mutations (n = 20) in the 120- and 200-mg gilteritinib dose groups, which would have been insufficient for our analyses. Moreover, the NGS assay that was used was specifically designed for the detection of FLT3 insertions that characterize FLT3-ITD mutations. This assay allows for exquisite sensitivity as the particular FLT3 insertion is unique for each patient, whereas a point mutation could be mistaken for noise due to inherent polymerase error. Currently, there is no available NGS assay with sufficient sensitivity (at least 1 × 10⁻⁴) to detect FLT3-TKD point mutations.

In conclusion, this is the first study that evaluated the long-term effect of FLT3-ITD mutation clearance after treatment with a FLT3 inhibitor in patients with FLT3 mut+ R/R AML. Gilteritinib was effective in inducing FLT3-ITD clearance and remission in these patients. Patients who achieved morphologic remission with FLT3-ITD clearance, defined as MRD negativity, showed a trend toward longer survival than those who achieved remission with persistence of the FLT3-ITD clone. Although the ELN does not currently recommend the use of FLT3-ITD mutations in the analysis of MRD due to the unstable nature of these mutations, assessment of mutation clearance in patients receiving these therapies will be an important determinant of response durability in patients who achieve remission as the use of highly specific agents against FLT3 and other leukemia targets becomes more prevalent in AML. Given that the goal of treatment is to achieve remission and prevent relapse, it will be important to determine the impact of gilteritinib therapy on mutation clearance in patients with newly diagnosed AML.

ACKNOWLEDGMENTS

This study was funded by Astellas Pharma, Inc. Medical writing/editorial support was provided by Kalpana Vijayan, PhD, and Elizabeth Hermans, PhD, from OPEN Health Medical Communications, Chicago, IL, and funded by the study sponsor.

DISCLOSURES

JK Altman reports institutional grant funding from Boehringer Ingelheim, Astellas, Agios, Celgene, Genetech, FujiFilm, and BioSight; review activities with Glycomimetics and the US lead PI for BioSight trial; advisory board participation with Astellas, Novartis, Syros, Jannsen Pharmaceuticals, Celgene, Immune Pharmaceuticals (did not accept payment), Bristol Myers Squibb, ASH, Cancer Expert Now, Agios, Theradex, AbbVie, and Daiichi Sankyo; and personal fees from Astellas. AE Perl reports personal fees and non-financial support from Astellas during the conduct of the study; institutional grant funding from Astellas, Bayer, BioMed Valley Discoveries, Daiichi Sankyo, Fujifilm, and Novartis; personal fees and other support from Daiichi Sankyo, Arog, Novartis, Jazz Pharmaceuticals, Takeda Oncology, AbbVie, NewLink Genetics, Asana Biosciences, and Seattle Genetics; and personal fees from Pfizer and Actinium Pharmaceuticals. JE Hill is an employee of Astellas; reports stock ownership in Ligacept, LLC; and has patents US 7862995, US 9051388, and US 9683222 issued. M Rosales and E Bahcecci are employees of Astellas. MJ Levis reports grant funding from Astellas during the conduct of the study, grant funding from Novartis and FujiFilm, and personal fees from Daiichi Sankyo.

AUTHOR CONTRIBUTIONS

JEH, EB, and MJL designed the research study; JEH performed the research; JKA, AEP, and JEH acquired the data; JEH, MR, EB, and MJL analyzed the data; JKA, AEP, JEH, MR, EB, and MJL interpreted the data; JEH drafted the manuscript; and JKA, AEP, JEH, MR, EB, and MJL critically reviewed and revised manuscript. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

Researchers may request access to anonymized participant-level data, trial-level data, and protocols from Astellas sponsored clinical trials at www.clinicalstudydatarequest.com.

For the Astellas criteria on data sharing, see: https://clinicalstudydatarequest.com/Study-Sponsors/Study-Sponsors-Astellas.aspx

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