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Pierre-Yves Dumas, Emmanuel Raffoux, Emilie Bérard, Sarah Bertoli, Marie-Anne Hospital, et al..
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ALFA/FILO. *Leukemia*, 2023, 37 (1), pp.91-101. 10.1038/s41375-022-01742-7 . hal-04244552

HAL Id: hal-04244552

<https://univ-rennes.hal.science/hal-04244552>

Submitted on 30 Nov 2023

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Gilteritinib activity in refractory or relapsed *FLT3*-mutated acute myeloid leukemia patients previously treated by intensive chemotherapy and midostaurin: a study from the French AML Intergroup ALFA/FILO.

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70 **Word count**

71 Abstract: 200

72 Text: 3833

73 Tables: 5

74 Supplementary Tables: 8

75 Supplementary Method: 1

76 Figures: 2

77 Supplementary Figures: 2

78

79 **Running title**

80 Real-world data in R/R *FLT3*-mutated AML treated by gilteritinib

81

82 **Keywords**

83 Acute myeloid leukemia, *FLT3* mutation, primary induction failure, relapse, gilteritinib, midostaurin,

84 tyrosine kinase inhibitors

Abstract

The real-world efficacy and safety of gilteritinib was assessed in an ambispective study that included 167 R/R *FLT3*-mutated AML patients. Among them, 140 received gilteritinib as single agent (cohort B), including 67 previously treated by intensive chemotherapy and midostaurin (cohort C). The main differences in patient characteristics in this study compared to the ADMIRAL trial were ECOG ≥ 2 (83.6% vs 16.6%), *FLT3*-TKD mutation (21.0% vs 8.5%), primary induction failure (15.0% vs 40.0%) and line of treatment (beyond 2nd in 37.1% vs 0.0%). The rates of composite complete remission, excluding those that occurred after hematopoietic stem cell transplantation (HSCT), were similar at respectively 25.4% and 27.5% in cohorts B and C. Median overall survival (OS) for these two groups was also similar at respectively 6.4 and 7.8 months. Multivariate analyses for prognostic factors associated with OS identified female gender (HR 1.61), adverse cytogenetic risk (HR 2.52) and allogenic HSCT after gilteritinib (HR 0.13). Although these patients were more heavily pretreated, these real-world data reproduce the results of ADMIRAL and provide new insights into the course of patients previously treated by intensive chemotherapy and midostaurin and beyond the 2nd line of treatment who can benefit from treatment in an outpatient setting.

Introduction

Internal tandem duplication (ITD) in the *FLT3* gene is one of the most frequent mutations found in acute myeloid leukemia (AML) ¹. *FLT3*-ITD is associated with poor prognosis ² and has emerged as a relevant therapeutic target ³. *FLT3*-ITD is usually conserved at relapse ⁴, even if prevalence decreases in patients who received midostaurin in front-line treatment ⁵, suggesting that *FLT3*-ITD AML-initiating cells are key targets for long-lasting remission ^{2,6}. Before the era of second-generation *FLT3* tyrosine kinase inhibitors (TKI), there was no standard approach to treat relapsed/refractory (R/R) AML beyond the need for enrollment into clinical trials. Commonly used therapeutic options included intermediate- or high-dose aracytine (I/HDAC)-based salvage chemotherapies, sequential reduced-intensity conditioning for allogeneic hematopoietic stem cell transplantation (HSCT) and best supportive care ⁷. In the subgroup of *FLT3*-mutated AML, the QUANTUM-R trial, which was designed specifically for R/R *FLT3*-ITD AML patients, demonstrated the superiority of quizartinib as single agent over the control arm with median overall survival (OS) at 6.2 months in the quizartinib arm and 4.7 months in the control arm. These results allowed approval in Japan but not in the US and EU. The ADMIRAL phase 3 trial, designed for both R/R *FLT3*-ITD and tyrosine kinase domain (-TKD) mutated AML patients, demonstrated the superiority of gilteritinib as single agent over the control treatment arm, which was determined by investigators prior to 2:1 randomization between mitoxantrone, etoposide, cytarabine (MEC) or fludarabine, cytarabine, granulocyte colony-stimulating factor, idarubicin (FLAG-Ida) or azacitidine (AZA) or low-dose aracytine (LDAC). In this trial, OS was significantly improved in the gilteritinib arm compared to the control arm with a hazard ratio (HR) of 0.64 (95%CI 0.49-0.83). Median OS was 9.3 months in the gilteritinib arm and 5.6 months in the control arm. Best response was noted at any post-baseline visit including the post-HSCT period, leading to composite complete remission (CRc) [complete remission (CR) + CR without hematological recovery (CRi) + CR with incomplete platelet recovery (CRp)] of 54.3% in the gilteritinib arm vs 21.8% in the control arm. When remissions that occurred after HSCT were excluded, the percentage of patients who had CR with full or partial

hematologic recovery was 26.3% in the gilteritinib group vs 15.3% in the chemotherapy group⁸. These results led to the approval of gilteritinib monotherapy in US and EU in patients with R/R *FLT3*-mutated AML and it is now a standard treatment in such situations. Nevertheless, in the ADMIRAL trial, only 5.7% of patients had been exposed to midostaurin as front line prior to gilteritinib⁹. Therefore, this pivotal trial underrepresented current patients previously exposed to midostaurin, since virtually all *FLT3*-mutated AML patients now receive midostaurin added to standard intensive chemotherapy as first-line therapy⁹.

Therefore, the objective of the current study was to describe the characteristics, treatments, and outcomes of R/R *FLT3*-mutated AML patients treated in a real-world setting by gilteritinib and to report results in patients who had previously received an intensive chemotherapy associated with midostaurin.

Methods

This non-interventional ambispective study included virtually all patients receiving gilteritinib in the French early access program between March 1st, 2019, and March 1st, 2021, in 37 centers. All patients ≥ 18 years with AML according to the WHO classification ¹⁰ who received gilteritinib, as single agent or in combination, for R/R AML with *FLT3* mutation were included. Newly diagnosed AML patients and patients who received gilteritinib out of the scope of its marketing approval such as post-HSCT maintenance in patients in CR1 after intensive chemotherapy were excluded. This study has been registered in ClinicalTrials (NCT05193448) and has been approved by ethics authorities (Comité de Protection des Personnes Ouest III) with approval number 2021-A00993-38. Cytogenetic risk classification was defined according to the United Kingdom Medical Research Council classification ¹¹. Response to treatment, relapse-free survival (RFS), event-free survival (EFS), cumulative incidence of relapse (CIR), and OS were defined according to the European LeukemiaNet (ELN) 2017 criteria ⁷. Primary refractory AML and relapse were defined according to ELN 2017 ⁷: the former was defined as a failure to achieve CR or CRi after one or two courses of induction chemotherapy; the latter was defined as bone marrow blasts $\geq 5\%$ or reappearance of blasts in the blood, or development of extramedullary disease. Serious adverse events (SAE) were defined according to FDA code. More details about Methods are available in Supplementary material.

Results

Study population at diagnosis and front-line treatment

A total of 167 patients (Cohort A) with *FLT3*-ITD and/or TKD mutated AML fulfilled the inclusion criteria. Among these 167 patients, 27 received gilteritinib associated with another drug and 140 received gilteritinib as single agent (Cohort B). Finally, among these 140 patients, 67 received gilteritinib in an R/R setting following a front-line strategy including intensive chemotherapy and midostaurin (Cohort C) (Supplementary Figure 1). Characteristics of patients in these three cohorts are described in Table 1. In cohort A, 73 (43.7%) patients were 65 years of age or older. The median white blood cell count (WBC) was $31.7 \times 10^9/L$ (IQR, 8.7-90.9) at diagnosis. There were 141 (84.4%) cases of *de novo* AML. Cohort A displayed an *FLT3*-ITD mutation in 104 (62.3%) patients and an *FLT3*-TKD mutation in 33 (19.8%), whereas 36 (21.6%) were *FLT3* wild type at diagnosis and acquired mutation later in the disease evolution. Finally, 6 (3.6%) patients had both the *FLT3*-ITD and TKD mutation at diagnosis. Median *FLT3*-ITD/wt ratio was 0.50 (IQR, 0.12-0.70 and range 0.01-1.90), and median size of ITD was 48 bp (IQR, 30-75 and range 3-408). Most patients (136, 81.4%) had an intermediate cytogenetic risk, and 84 (50.3%) had an *NPM1* co-mutation.

In cohort A, 153 (91.6%) patients received front-line treatment by intensive chemotherapy, 54 (35.3%) a daunorubicin-based “3+7” and 86 (56.2%) an idarubicin-based “3+7”. This intensive chemotherapy was associated with midostaurin in 87 (56.9%) patients, whereas 3 (2.0%) other patients received quizartinib or ponatinib in clinical trials. Median duration of targeted therapy added to intensive chemotherapy was 120.5 days (IQR, 65.0-287.0 and range 9.0-456.0) and 33 patients received maintenance treatment after CR1/CRi1. Among the 153 patients who received an intensive chemotherapy, 125 (81.7%) were in CR1/CRi1 after 1 or 2 courses of intensive chemotherapy. Furthermore, 14 (8.4%) patients received front-line treatment by hypomethylating agents (HMA), including 3 (21.4%) associated with midostaurin. Among the 14 patients who received HMA, 3 (21.4%)

were in CR1/CRi1, and median number of cycles of HMA before best response was 5 (IQR, 2.0-6.0 and range 1.0-8.0). Twenty-two (13.2%) patients received an allogenic HSCT in CR1/CRi1. Finally, 128 (76.6%) patients in CR1/CRi1 relapsed. The median time to relapse from CR/CRi was 6.6 months (IQR, 3.9-11.8; range 0.1-101.9), and 3.4 months (IQR, 1.8-5.9; range 1.6-59.9) in the 22 patients who received HSCT in CR1/CRi1. Lastly, before gilteritinib, 75 (44.9%) patients received a second-line treatment, 32 (19.2%) patients received a third-line treatment, and 9 (5.4%) patients received a fourth-line treatment. A total of 42 (25.1%) patients received an allogenic HSCT before gilteritinib. Characteristics of patients and results of front-line treatment for cohort B (n=140) and C (n=67) are described in Table 2, with similar results in the three cohorts.

Characteristics of patients at gilteritinib initiation

The characteristics of the three cohorts at gilteritinib initiation are described in Table 3. In cohort A, 72 (51.4%) were 65 years of age or older. The median WBC was $3.8 \times 10^9/L$ (IQR, 1.9-14.5). Sixty-eight (40.7%) did not have a new karyotypic analysis at relapse, 66 (39.5%) had a karyotype similar to the diagnosis and 27 (16.2%) a different one. Thirty-six (21.5%) patients unmutated at diagnosis acquired an *FLT3* mutation: 27 (75.0%) patients acquired a *FLT3*-ITD mutation and 9 (25.0%) patients a *FLT3*-TKD mutation. Overall, 36 patients among 167 (21.5%) had a single TKD mutation. Finally, at gilteritinib initiation, 25 (15.0%) patients were refractory to front-line treatment, 41 (24.6%) were in 1st relapse \leq 6 months from CR1/CRi1, 39 (23.4%) were in 1st relapse $>$ 6 months from CR/CRi, 17 (10.2%) were refractory to the treatment of the 1st relapse and 45 (26.9%) were beyond the 1st relapse. Patients' characteristics were similar in the three cohorts but among 67 patients in cohort C who received gilteritinib as single agent following a front-line strategy including intensive chemotherapy and midostaurin, only 5 (7.5%) were in primary induction failure. Lastly, among 167 patients in cohort A, 140 received gilteritinib as single agent (Cohort B) and 27 in combination: 17 (10.2%) with an HMA, 6 (3.6%) with venetoclax, and 4 (2.4%) with intensive chemotherapy, low-dose

cytarabine or other treatments. In cohorts B and C, 29 (21.2%) and 10 (15.2%) patients, respectively, received hydroxyurea with gilteritinib for a median duration of 14.0 days (IQR 5.0-37.0, range 1.0-96.0) and 10.0 days (IQR 5.0-37.0, range 5.0-96.0), respectively.

Response to treatment

Apart from remissions that occurred after HSCT, the response to gilteritinib in the three cohorts is described in Table 4. Among 167 patients in cohort A, the best response to treatment was CR/CRi for 38 (24.4%) patients and CRc (CR+CRi+CRp) for 41 (26.3%). Among 140 patients who received gilteritinib as single agent in cohort B, the best response to treatment was CR/CRi for 30 (23.1%) patients, CRc (CR+CRi+CRp) for 33 (25.4%), overall response rate (ORR) [CRc+ partial response (PR)] for 40 (30.8%) patients, and morphological leukemia-free state (MLFS) for 9 (6.9%) patients. Sixty-eight (52.4%) patients were in failure and 6 (4.6%) died before evaluation. In multivariate analysis, no prognostic factor was independently and significantly associated with response among age, ECOG at gilteritinib initiation, secondary AML, sex, white blood cell count (WBC) at gilteritinib initiation, cytogenetic risk, ELN 2017 classification, *FLT3*-ITD, *FLT3*-TKD, ITD/wt ratio, previous HSCT before gilteritinib, prior treatment with midostaurin, and prior treatment with FLT3-TKI. Similar results were observed in cohort C with a best response to treatment that was CR/CRi for 16 (25.9%) patients, CRc for 17 (27.5%) patients, ORR for 21 (34.0%) patients and MLFS for 3 (4.8%) patients. Thirty-one (50.0%) patients were in failure and 3 (4.8%) died before evaluation. Time to the best response was 1.9 months (IQR, 1.0-2.9; range 0.1-15.1) in cohort B and 2.1 months (IQR, 1.1-3.2; range 0.1-8.4) in cohort C. Response to gilteritinib in subgroups defined as per AML phase at gilteritinib initiation are described in Supplementary Table 1. Briefly, among 140 patients who received gilteritinib as single agent, CRc rates were 5.0%, 43.3%, 33.3% and 18.9% in following subgroups : refractory after front-line (N=22), 1st relapse ≤ 6 months from CR/CRi (N=32), 1st relapse > 6 months from CR1/CRi1 (N=30) and refractory after 1st relapse and beyond (N=56), respectively. Finally, 32 (19.2%), 25 (17.9%) and 15 (22.4%)

patients received an allogenic HSCT after gilteritinib, in CRc for 21 (65.6%), 17 (68.0%) and 11 (73.3%) patients in cohorts A, B and C, respectively (Supplementary Table 2). In cohort A, 12 (37.5%) patients resumed gilteritinib after HSCT as maintenance strategy after a median time from HSCT of 1.6 months (IQR 1.1-3.1, range 0.7-10.2) and for a period of 1.9 months (IQR 1.2-7.0, range 1.0-12.0). Among patients who received maintenance with gilteritinib, only 1 (8.3%) underwent an acute graft versus host disease (GVHD) after 61 days of treatment and 2 (16.7%) a chronic GVHD after 43 and 69 days of treatment.

In subgroup of patients having previously received induction chemotherapy in front-line and then treated with gilteritinib as single agent, we compared the subgroup of patient who received “3+7” and midostaurin in front-line, previously identified as Cohort C and those who received “3+7” in front-line without midostaurin nor other FLT3-TKI (N=56/126). Baseline characteristics, front-line treatment, relapses, characteristics at gilteritinib initiation and response to gilteritinib have been described in Supplementary Tables 3 to 6. Briefly, CRc/ORR were at 27.5%/34.0% and 28.3%/30.2% in patients treated by single agent gilteritinib after prior exposure to “3+7” with midostaurine (N=67) and without any TKI (N=56), respectively (p=NS) (Table 4 and Supplementary Table 6 and 7). In others subgroup, among cohort B: CRc was at 0% in adverse cytogenetic risk vs 27.1% in favorable/intermediate cytogenetic risk (p=0.04); CRc was at 30.5% in patients that received previous front- or subsequent-line of treatment with midostaurin vs 25.0% in patients that received previous front- or subsequent-line of treatment with FLT3-TKI other than midostaurin (p=NS); CRc was at 29.4% in *FLT3*-ITD subgroup vs 15.4% in *FLT3*-TKD subgroup (p=NS) (Supplementary Table 7). Finally, among the whole cohort A, CRc was at 25.4% in gilteritinib single agent vs 30.8% in cohort of patients who received gilteritinib associated with another drug (p=NS) (Supplementary Table 7).

Safety

Among 98 patients in cohort B with a documented ECG before gilteritinib initiation and who received gilteritinib as single agent, 87 (88.8%) and 11 (11.2%) patients had QTcF <450 ms or >450 but <500 ms, respectively. Finally, 132 (94.3%) patients started gilteritinib at 120 mg per day, 103 (73.6%) did not receive any dose modification, 12 (8.6%) had their posology increased to 200 mg per day, 15 (10.7%) had it reduced to 80 mg/day, and 10 (7.1%) had other dose modifications. These modifications were made after a median time of 43 days (IQR 14-72, range 1-368), owing to QTcF modification in 2 patients (5.4%), hematological toxicity in 12 (32.4%), lack of efficacy in 13 (35.1%) and other reasons in 10 (27.0%). Reasons for gilteritinib discontinuation were hematological toxicity in 4 (3.0%) patients or other adverse event (AE) in 8 (6.0%) patients, but not for QTcF prolongation. Eighty-nine (66.9%) patients discontinued treatment because of lack of efficacy or death, whereas 18 (13.5%) patients discontinued for HSCT and 14 (10.6%) patients for other reasons. Grade ≥ 3 hematological and non-hematological adverse events are described in Table 5. A total of 6 (4.3%) patients experienced a differentiation syndrome and 1 (0.7%) a tumor lysis syndrome. No posterior reversible encephalopathy syndrome nor acute pancreatitis was noted during the study.

Thirty-nine patients (28.3%) experienced a serious AE (SAE) during gilteritinib treatment: infections in 22 (56.4%), hemorrhage in 1 (2.6%), cardiovascular in 4 (10.3%), hepatic in 4 (10.3%), differentiation syndrome in 2 (5.2%), and other SAE in 6 (15.4%). In terms of health care resource consumption, median duration of hospitalization during the first 6 months of treatment was 9.0 days (IQR 0-29, range 0-117) with transfusion of 7 RBC units (IQR 0-14, range 0-54) and 5 platelet units (IQR 0-13.5, range 0-56).

Outcomes

After a median FU of 13.8 months (IQR, 9.9-17.7), the median OS of the whole study cohort A was 6.4 months (IQR, 3.2-14.3) (Figure 1A). Among 140 patients in Cohort B who received gilteritinib as single agent and after a median FU at 14.5 months (IQR, 9.9-17.7), median RFS, EFS, and OS were

9.8 months (IQR, 2.8–not reached [NR]), 3.9 months (IQR, 1.6–11.3), and 6.4 months (IQR, 3.2–14.7) (Figure 1B), respectively. Moreover, 12-month RFS, EFS and OS were 37.3% (95%CI 18.2-56.5), 23.6% (95%CI 16.5-31.5) and 31.4% (95%CI 22.4-39.7), respectively. Multivariate analyses for factors significantly and independently associated with OS found female gender (HR 1.61, 95%CI 1.07-2.42, $p=0.02$), adverse cytogenetic risk (HR 2.52, 95%CI 1.24-5.13, $p=0.01$) (Figure 2A and B) and HSCT after gilteritinib (HR 0.13, 95%CI 0.05-0.37, $p<0.0001$). Among 33 patients in CRc in cohort B, 15 (45.5%) relapsed. Early death \leq day 30 occurred in 7 (5.0%) patients and \leq day 60 in 18 (12.9%). Among 67 patients in cohort C who received gilteritinib in an R/R setting following a front-line strategy including intensive chemotherapy and midostaurin, and after a median FU at 14.5 months (IQR, 9.2-17.7), median EFS and OS were 3.9 months (IQR, 2.4–15.7) and 7.8 months (IQR, 3.2-20.8) (Figure 1C), respectively. Moreover, 12-month EFS and OS were 31.0% (95%CI 20.1-42.4) and 38.3% (95%CI 26.0-50.4), respectively. Among 17 CRc patients in cohort C, 7 (41.2%) patients relapsed. Early death \leq day 30 and \leq day 60 occurred in 1 (1.5%) and 10 (14.9%) patients, respectively. Median OS and EFS in various subgroups are described in Supplementary Table 8.

We also compared outcomes in Cohort C and in patients who received “3+7” in front-line without midostaurin nor other FLT3-TKI (N=56/126) for OS and EFS that are quite similar and are shown in Figure 2C and D. We also compared OS and EFS depending on treatment added to gilteritinib, showing same median OS and EFS (Supplementary Figure 2A and B). Moreover, patients that were previously exposed to FLT3-TKI other than midostaurin in front- or subsequent-line of treatment showed a shorter OS with a 1-year OS at 18.3% (IC95% 6.0-35.9) vs 43.0% (IC95% 29.8-55.5) ($p<0.05$) and a trend for shorter EFS with a 1-year EFS at 14.4% (IC95% 3.9-31.4) vs 31.2% (IC95% 19.6-43.4) ($p=0.11$) (Supplementary Figure 2C and D). Finally, *FLT3* mutation (ITD vs TKD) showed just a trend to better OS and EFS for *FLT3*-ITD patients treated by gilteritinib, even if 1-year OS was at 36.6% (IC95% 26.4-46.8) in *FLT3*-ITD subgroup vs 17.1% (IC95% 5.9-33.3) in *FLT3*-TKD subgroup ($p=0.09$) (Figure 2E and F).

***FLT3* mutation persistence at relapse after Gilteritinib treatment**

Among the 140 patients of cohort B, 33 (25.4%) were in CRc after gilteritinib treatment, and among them, at the time of data analysis, 15 (46.3%) relapsed. Among these 15 patients, 14 have had a new *FLT3*-ITD evaluation and solely 10 (71.4%) still displayed a *FLT3*-ITD mutation with a median *FLT3*-ITD/wt ratio at 0.50 (IQR, 0.27-0.56 and range 0.08-0.80), and median size of ITD was 40 bp (IQR, 33-45 and range 2-66). One had also a *FLT3*-TKD D835Y mutation. Finally, among the 67 patients of cohort C, 17 (27.5%) were in CRc after gilteritinib treatment, and among them, at the time of data analysis, 7 (41.2%) relapsed. Among these 7 patients, 6 have had a new *FLT3*-ITD evaluation and solely 4 (66.6%) still displayed a *FLT3*-ITD mutation with a median *FLT3*-ITD/wt ratio at 0.50 (IQR, 0.29-0.53 and range 0.08-0.56), and median size of ITD was 36 bp (IQR, 27-54 and range 24-66). None still have a *FLT3*-TKD mutation.

Discussion

In the current study, we described the characteristics and outcomes of R/R *FLT3*-mutated AML patients in order to evaluate the efficacy and safety of gilteritinib in a real-world setting, especially for patients previously treated by intensive chemotherapy and midostaurin. The main differences in patient characteristics in this study vs the ADMIRAL trial were ECOG ≥ 2 (83.6% vs 16.6%), *FLT3*-TKD mutation (21.5% vs 8.5%), primary induction failure (15.0% vs 40.0%) and line of treatment (beyond 2nd in 37.1% vs 0.0% since such patients were excluded from ADMIRAL). Broadly, R/R *FLT3*-mutated AML patients included in this study were in poor general condition, less frequently in primary induction failure but much more heavily pretreated.

Despite such discrepancies, among the 140 patients who received gilteritinib as single agent, the CRc rate was 25.4% in our cohort compared to 26.3% in the gilteritinib arm of the ADMIRAL trial, when remissions that occurred after HSCT were excluded. Median OS was 6.4 months in the current study vs 9.3 months in the gilteritinib arm of the ADMIRAL trial, and 1-year OS was 37.1% vs 31.4%, respectively. The real-life safety profile showed 40.0% of grade ≥ 3 anemia, as in ADMIRAL trial, but twice as many cases of grade ≥ 3 thrombocytopenia. As previously observed, QTcF prolongation > 450 ms were rare and without clinical impact. Discontinuation of gilteritinib due to lack of efficacy or death was also comparable: 64% in the ADMIRAL study and 67% in the current study.

The most important finding concerns previous exposure to intensive chemotherapy associated with midostaurin. As a reminder, the RATIFY trial randomized 717 patients with newly diagnosed *FLT3*-mutated AML to receive conventional "3+7" chemotherapy followed by HDAC consolidations with or without midostaurin, followed by 1 year of maintenance. The combination significantly increased 4-year OS by 51.4% vs 44.3%, and prolonged median OS to 74.7 vs 25.6 months⁹. To date, another study addressed the question of gilteritinib efficacy in patients who previously received *FLT3*-TKI (midostaurin, sorafenib and others) in 11 US centers. However, one third of patients received gilteritinib associated with other treatments so some results were difficult to interpret. Median OS was

7.0 months in the whole cohort and 7.8 months in the subgroup of patients who previously received intensive chemotherapy and midostaurin ¹², so the results of this subgroup are reassuring since they are similar to those of other patients. Another recent study included 33 patients from the CHRYSALIS phase 1/2 trial ¹³ who previously received sorafenib and 33 patients from the ADMIRAL phase 2 trial who previously received midostaurin or sorafenib in 42% and 58% cases, respectively. Median OS was 7.2 months and CRc rates were quite similar to those of patients who did not receive prior TKI ¹⁴. To date, our study provides the largest cohort of patients treated by gilteritinib as single agent in real life and the largest cohort of patients receiving gilteritinib after a prior front-line treatment by “3+7” and midostaurin, which is currently the gold standard.

Surprisingly, multivariate analyses for factors significantly and independently associated with OS found female gender associated with much more expected factors such as adverse cytogenetic risk and HSCT after gilteritinib. In the specific subgroup of adverse cytogenetic risk, we also showed that response rates were dramatically decreased. There are conflicting reports in the literature suggesting that gender impacts survival of AML patients. Recently, a specific study has been done in an attempt to resolve this issue, showing that females patients with AML have a greater OS compared with males, irrespective of age at diagnosis. Interestingly, gender did not influence outcome in patients with a *FLT3*-ITD mutation, also suggesting a link between *FLT3* mutation, gender and response to treatment¹⁵. On the other hand, sex-biased expression levels of enzymes or transporters in liver or kidney leading to different pharmacokinetics are described for most common anti-cancer drugs including TKI but no data are currently available for gilteritinib. Noteworthy, in ADMIRAL study, female gender was associated with a significant HR for death at 0.57 (0.40-0.82) so the opposite of our results. We have no clear explanation for this intriguing point, which should be interpreted with caution and should prompt additional investigations.

The main limitation of the present study is obviously its ambispective nature. We cannot exclude selection bias, although we included virtually all patients who received gilteritinib in France during the period of accrual in 37 centers that cover a large proportion of the French population.

375 Second, there may also be a measurement bias in remission rates based on real-world data vs an
376 experimental arm of a prospective trial, so we presented results as an intention-to-treat cohort
377 including patients not evaluated before death or treatment discontinuation. Finally, real-world data
378 reproduced results from the ADMIRAL study. Despite some discrepancies between patient
379 characteristics, these results provide a care pathway for adverse prognosis patients in an outpatient
380 setting. Despite preclinical data suggesting its efficacy to overcome some resistance mechanisms ^{16,17},
381 it has been shown that mutational landscape ^{18,19} and microenvironment ²⁰ allow AML blasts to resist
382 to gilteritinib, precluding long-lasting remission or cure of *FLT3*-mutated AML by FLT3-TKI as single
383 agent. Gilteritinib is therefore an interesting therapeutic pillar whose results can still be greatly
384 improved, in association with intensive chemotherapy ²¹, hypomethylating agents ²², BH3 mimetics ^{23,24}
385 and others ²⁵.

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Figure Legends

Figure 1. Overall survival from gilteritinib initiation: A) in cohort A (n = 167), B) in cohort B (n = 140), C) in cohort C (n = 67)

Figure 2. Overall survival (left panel) and event free survival (right panel) from gilteritinib initiation in cohort B of patient treated with single agent gilteritinib: A/B) Adverse cytogenetic risk (n = 9) vs Favorable/intermediate cytogenetic risk (n = 131), C/D) Prior exposure to “3+7” + midostaurin (n = 67) vs prior exposure to 3+7 without midostaurin nor other FLT3 tyrosine kinase inhibitor (n = 56), E/F) *FLT3*-ITD without *FLT3*-TKD mutated patients (n = 100) vs *FLT3*-TKD without *FLT3*-ITD mutated patients (n=28).

474 **Disclosure**

475

476 Pierre-Yves Dumas: Daiichi-Sankyo, Jazz Pharmaceutical, Astellas, Abbvie, Celgene, Janssen

477 Emmanuel Raffoux: Daiichi-Sankyo, Astellas, Abbvie, Celgene, Pfizer

478 Emilie Berard: no competing interest

479 Sarah Bertoli: Jazz Pharmaceuticals, Daiichi-Sankyo, Sanofi, Astellas and BMS

480 Marie-Anne Hospital: no competing interest

481 Maël Heiblig: Astellas, Pfizer, Abbvie, Jazz Pharmaceuticals, Servier

482 Yohann Desbrosses: Jazz Pharmaceuticals, Abbvie, Celgene, Novartis

483 Caroline Bonmati: no competing interest

484 Cécile Pautas: Abbvie, BMS

485 Juliette Lambert: Pfizer, Astellas, Abbvie

486 Corentin Orvain: Novartis

487 Anne Banos: no competing interest

488 Florence Pasquier: no competing interest

489 Pierre Peterlin: Daiichi-Sankyo, Jazz Pharmaceutical, Astellas, Abbvie, BMS

490 Tony Marchand: Jazz Pharmaceutical, Servier

491 Madalina Uzunov: no competing interest

492 Jamilé Frayfer: no competing interest

493 Pascal Turlure: Daiichi-Sankyo

494 Thomas Cluzeau: Astellas, Novartis and institution: Novartis, Astellas, Arog

495 Eric Jourdan: Novartis, Abbvie, BMS

496 Chantal Himberlin: no competing interest

497 Emmanuelle Tavernier: Abbvie, BMS

498 Alban Villate: no competing interest

499 Stephanie Haiat: no competing interest

500 Marie-Lorraine Chretien: no competing interest

501 Martin Carre: Astellas, BMS, Jazz pharmaceutical

502 Sylvain Chantepie: no competing interest

503 Ioana Vaida: no competing interest

504 Mathieu Wemeau: Abbvie, AOP Orphan, BMS, Gilead, Novartis

505 Safia Chebrek: no competing interest

506 Gaelle Guillerm: no competing interest

507 Romain Guièze: Abbvie, Janssen, Beigene, Astrazeneca, Roche, Amgen.

508 Houria Debarri: no competing interest

509 Eve Gehlkopf: no competing interest

510 Kamel Laribi: Outside this work, KL received Grants from Novartis, Takeda, Jansen, Abbvie, and

511 personal fees from Novartis, Takeda, Abbvie, Iqone, Astra Zeneca, and Beigene.

512 Ambroise Marcais: no competing interest

513 Alberto Santagostino: no competing interest

514 Marie-Christine Béné: no competing interest

515 Ariane Mineur: no competing interest

516 Arnaud Pigneux: Grant/Research Support: Astellas, Roche ; Speaker's Bureau : Astellas, AbbVie, Gilead,

517 Pfizer, Roche, Sanofi ; Consultant : Jazz, AbbVie, Agios, BMS, Gilead, Novartis, Pfizer, Roche, Takeda

518 Hervé Dombret: Honoraria/consulting: Abbvie, Amgen, Astellas, Celgene-BMS, Daiichi Sankyo, Incyte,

519 Jazz Pharmaceuticals, Pfizer, Servier; research funding: Amgen, Astellas, Celgene-BMS, Incyte, Jazz

520 Pharmaceuticals, Pfizer

521 Christian Récher: Research grants from AbbVie, Amgen, Novartis, BMS-Celgene, Jazz Pharmaceuticals,

522 Agios, Chugai, MaaT Pharma, Astellas, Roche, Daiichi-Sankyo and Iqvia; an advisory role for AbbVie,

523 Janssen, Jazz Pharmaceuticals, Novartis, Celgene, Otsuka, Astellas, Daiichi-Sankyo, Macrogenics, Pfizer.

524 Roche, Servier and Takeda

Acknowledgments

We would like to thank Astellas for their financial support enabling e-CRF. Astellas was not involved in data collection, analysis or in the writing of this article.

Contributions

Conceptualization, PYD ; Methodology, PYD, EB ; Patients care, PYD, ER, SB, MAH, MH, YD, CB, CP, JL, CO, AB, FP, PP, TM, MU, JF, PT, TC, EJ, CH, ET, AV, SH, MLC, MC, SC, IV, MW, SC, GG, RG, HD, EG, KL, AM, AS, AP, HD, CR ; Collected the data, AM, MCB ; Analyzed data, PYD, EB ; Writing—original draft, PYD ; Revised the manuscript, CR, JL, PP, MH, CO, RG ; Review and editing, PYD, EB, CR ; Funding acquisition, PYD, AM.

Data availability

Some de-identified data will be shared with other researchers upon reasonable request to the corresponding authors (pierre-yves.dumas@u-bordeaux.fr). The sharing will require a detailed proposal to the study investigators, and a data transfer agreement must be signed.

Table 1. Baseline characteristics at AML diagnosis

	Overall study n = 167 (100%) Single agent n=140 Combination n=27	Single agent gilteritinib n = 140 (100%) No prior mido n=70 Prior mido n=70	Single agent gilteritinib and prior exposure to “3+7+mido” n = 67 (100%)
Age (years) at diagnosis			
Median (IQR)	63.4 (52.4-70.1)	63.9 (52.6-70.4)	62.5 (52.1-70.1)
Range	18.2-84.8	20.8-84.8	23.5-77.8
Gender: n (%)			
Male	88 (52.7)	76 (54.3)	38 (56.7)
Female	79 (47.3)	64 (45.7)	29 (43.3)
ECOG at diagnosis: n (%)			
0-1	29 (19.7)	24 (18.9)	11 (17.7)
≥ 2	118 (80.3)	103 (81.1)	51 (82.3)
WBC at diagnosis (× 10⁹/L)			
Median (IQR)	31.7 (8.7-90.9)	28.7 (8.2-80.6)	37.0 (12.8-101)
Range	0.9-348.6	0.9-348.6	0.95-348.6
Blood blast at diagnosis (%)			
Median (IQR)	55.0 (21.0-80.0)	53.0 (21.0-79.0)	60.0 (37.0-80.0)
Range	0.0-98.0	0.0-98.0	1.0-97.0
BM blast at diagnosis (%)			
Median (IQR)	72.5 (48.0-87.0)	72.0 (48.0-86.0)	75.0 (55.5-88.0)
Range	0.0-98.0	0.0-98.0	0.0-95.0
AML status: n (%)			
<i>De novo</i>	141 (84.4)	115 (82.1)	56 (83.6)
Secondary AML ^a	26 (15.6)	25 (17.9)	11 (16.4)
Cytogenetics risk: n (%) ^b			
Favorable	5 (3.0)	4 (2.9)	2 (3.0)
Intermediate	136 (81.4)	119 (85.0)	56 (83.6)
Adverse	12 (7.2)	9 (6.4)	4 (6.0)
Failure	14 (8.4)	8 (5.7)	5 (7.5)
ELN 2017 prognosis: n (%)			
Favorable	23 (13.8)	18 (12.9)	6 (9.0)
Intermediate	112 (67.1)	96 (68.6)	47 (70.1)
Adverse	25 (15.0)	20 (14.3)	12 (17.9)
Unknown	7 (4.2)	6 (4.3)	2 (3.0)
FLT3 mutation: n (%) ^c			
FLT3-ITD	104 (62.3)	86 (61.4)	53 (79.1)
FLT3-TKD	33 (19.8)	26 (18.6)	13 (19.4)
Both	6 (3.6)	4 (2.9)	3 (4.5)
No mutation ^d	36 (21.6)	32 (22.9)	4 (6.0)
FLT3 ratio ITD/wt: n (%)			
< 3%	5 (5.3)	4 (5.1)	2 (4.1)
3–25%	28 (29.8)	24 (30.8)	19 (38.8)
26%–50%	16 (17.0)	14 (17.9)	8 (16.3)
> 50%	45 (47.9)	36 (46.2)	20 (40.8)
NPM1: n (%)			
Mutation	84 (50.3)	67 (47.9)	33 (49.3)
No mutation	77 (46.1)	67 (47.9)	33 (49.3)

Mido, midostaurin; ECOG, performance status; WBC, white blood cells; AML, acute myeloid leukemia; ELN, European LeukemiaNet; ITD, internal tandem duplication; wt, wild type; IQR, interquartile range.

^a non-*de novo* AML (12 post MDS, 2 post MPN, 3 post CMML and 9 therapy-related)

^b according to MRC 2010 classification

^c one patient can be included in several subgroups, patients *FLT3*wt at diagnosis acquired *FLT3* mutation later in disease history

^d 4 patients received midostaurin as front line treatment with intensive chemotherapy in clinical trial for *FLT3*wt AML

Table 2. Front-line treatment, relapses and subsequent lines of treatment before gilteritinib

	Overall study n = 167 (100%) Single agent n=140 Combination n=27	Single agent gilteritinib n = 140 (100%) No prior mido n=70 Prior mido n=70	Single agent gilteritinib and prior exposure to “3+7+mido” n = 67 (100%)
Front-line treatment: n (%)			
Intensive chemotherapy	153 (91.6)	126 (90.0)	67 (100.0)
Hypomethylating agent	14 (8.4)	14 (10.0)	-
Type of ICT: n (%)			
Daunorubicin-based 3+7	54 (35.3)	44 (34.9)	21 (31.3)
Idarubicin-based 3+7	86 (56.2)	69 (54.8)	42 (62.7)
GO-based 3+7	5 (3.3)	5 (4.0)	1 (1.5)
CPX-351	2 (1.3)	2 (1.6)	1 (1.5)
Other	6 (3.9)	6 (4.8)	2 (3.0)
FLT3-TKI added to ICT: n (%)^a			
None	63 (41.1)	70 (50.0)	-
Midostaurin	87 (56.9)	67 (47.9)	67 (100.0)
Other	3 (2.0)	3 (2.1)	-
FLT3-TKI added to HMA: n (%)^b			
None	10 (71.4)	10 (71.4)	-
Midostaurin	3 (21.4)	3 (21.4)	-
Other	1 (7.2)	1 (7.2)	-
Response to front-line ICT: n (%)			
CR/CRi in 1 course	111 (72.5)	93 (73.8)	48 (71.6)
CR/CRi in 2 courses	14 (9.2)	12 (9.5)	6 (9.0)
Failure	28 (18.3)	21 (16.7)	13 (19.4)
Response to front-line HMA: n (%)			
CR/CRi	3 (21.4)	3 (21.4)	-
Partial response	2 (14.3)	2 (14.3)	-
Stable disease	2 (14.3)	2 (14.3)	-
Failure	7 (50.0)	7 (50.0)	-
Relapses after front-line treatment: n (%)			
Bone marrow	128 (76.6)	108 (77.1)	54 (80.6)
MRD	108 (84.4)	91 (84.3)	44 (81.5)
MRD	18 (14.1)	16 (14.8)	10 (18.5)
Extramedullary	2 (1.5)	1 (0.9)	0 (0.0)

Mido, midostaurin; ICT, intensive chemotherapy; GO, gemtuzumab ozogamycin, AML, acute myeloid leukemia; TKI, tyrosine kinase inhibitor; HMA, hypomethylating agent; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; MRD, measurable residual disease; IQR, interquartile range.

^a the 3 patients classified as “other” received quizartinib (n=2) and ponatinib (n=1)

^b among 14 patients who received HMA, 3 patients received a regimen with Venetoclax, the patient classified as “other” received sorafenib (n=1)

Table 3. Characteristics of patients at gilteritinib initiation

	Overall study n = 167 (100%) Single agent n=140 Combination n=27	Single agent gilteritinib n = 140 (100%) No prior mido n=70 Prior mido n=70	Single agent gilteritinib and prior exposure to “3+7+mido” n = 67 (100%)
Age (years) at gilteritinib initiation:			
Median (IQR)	64.9 (53.4-71.5)	65.2 (53.8-72.1)	63.2 (53.3-71.7)
Range	19.1-86.1	23.1-86.1	25.4-78.5
ECOG at gilteritinib initiation: n (%)			
0-1	26 (16.4)	19 (14.1)	10 (15.6)
≥ 2	133 (83.6)	116 (85.9)	54 (84.4)
WBC at gilteritinib initiation ($\times 10^9/L$)			
Median (IQR)	3.8 (1.9-14.5)	3.8 (1.9-13.9)	4.1 (1.9-16.8)
Range	0.2-210.0	0.2-210.0	0.3-210.0
ANC at gilteritinib initiation ($\times 10^9/L$)			
Median (IQR)	1.2 (0.4-2.9)	1.1 (0.4-2.7)	1.2 (0.4-2.5)
Range	0.0-38.3	0.0-38.3	0.0-22.9
Hb at gilteritinib initiation (g/dL)			
Median (IQR)	9.9 (8.6-11.1)	9.8 (8.6-10.9)	10.2 (9.4-11.5)
Range	5.5-15.5	5.5-15.5	6.0-15.5
Pt at gilteritinib initiation ($\times 10^9/L$)			
Median (IQR)	49 (21-104)	43 (20-93)	50 (24-113)
Range	1-551	1-551	2-319
Blood blast at gilteritinib initiation (%)			
Median (IQR)	17.6 (0.0-58.0)	16.2 (0.0-52.0)	15.7 (0.0-60.0)
Range	0.0-99.0	0.0-99.0	0.0-94.0
BM blast at gilteritinib initiation (%)			
Median (IQR)	41.0 (9.0-75.0)	41.0 (8.0-77.0)	35.0 (7.0-77.0)
Range	0.0-96.0	0.0-96.0	0.0-96.0
AML phase at gilteritinib initiation: n (%)			
Refractory after front-line treatment	25 (15.0)	22 (15.7)	5 (7.5)
1 st relapse ≤ 6 months from CR/CRI1	41 (24.6)	32 (22.9)	20 (29.9)
1 st relapse > 6 months from CR/CRI1	39 (23.4)	30 (21.4)	20 (29.9)
Refractory after 1 st relapse	17 (10.2)	15 (10.7)	6 (9.0)
Beyond the 1 st relapse	45 (26.9)	41 (29.3)	16 (23.9)
Treatment associated with gilteritinib			
None	140 (83.8)	140 (100)	67 (100)
Intensive chemotherapy	2 (1.2)	-	-
HMA	17 (10.2)	-	-
LDAC	1 (0.6)	-	-
Venetoclax	6 (3.6)	-	-
Other	1 (0.6)	-	-

Mido, midostaurin; ECOG, performance status; WBC, white blood cells; ANC, absolute neutrophil count; Hb, hemoglobin; Pt, platelets; AML, acute myeloid leukemia; BM, bone marrow; IQR, interquartile range; CR, complete remission; CRI, complete remission with incomplete hematologic recovery; HMA, hypomethylating agent; LDAC, low-dose cytarabine

Table 4. Response to gilteritinib

	Overall study n = 167 (100%) Single agent n=140 Combination n=27	Single agent gilteritinib n = 140 (100%) No prior mido n=70 Prior mido n=70	Single agent gilteritinib and prior exposure to “3+7+mido” n = 67 (100%)
Best response: n (%)			
CR	24 (15.4)	22 (16.9)	12 (19.4)
CRi	14 (9.0)	8 (6.2)	4 (6.5)
CRp	3 (1.9)	3 (2.3)	1 (1.6)
PR	10 (6.4)	7 (5.4)	4 (6.5)
Combined criteria for best response: n (%)			
CR/CRi	38 (24.4)	30 (23.1)	16 (25.9)
CRC ^a	41 (26.3)	33 (25.4)	17 (27.5)
ORR ^b	51 (32.7)	40 (30.8)	21 (34.0)
MLFS	13 (8.3)	9 (6.9)	3 (4.8)
Failure ^c	75 (48.1)	68 (52.4)	31 (50.0)
No evaluation ^d	9 (5.8)	7 (5.4)	4 (6.5)
Deceased before evaluation	8 (5.1)	6 (4.6)	3 (4.8)
WBC at best response ($\times 10^9/L$)			
Median (IQR)	2.6 (1.2-5.1)	3.0 (1.4-5.6)	3.4 (1.5-6.1)
Range	0.1-54.1	0.1-54.1	0.1-30.8
ANC at best response ($\times 10^9/L$)			
Median (IQR)	1.1 (0.3-2.6)	1.2 (0.3-2.7)	1.5 (0.4-2.8)
Range	0.0-23.5	0.0-23.6	0.0-9.0
Hb at best response (g/dL)			
Median (IQR)	9.7 (8.6-10.8)	9.7 (8.6-10.8)	9.5 (8.6-11.1)
Range	6.8-78.0	6.9-78.0	7.3-78.0
Pt at best response ($\times 10^9/L$)			
Median (IQR)	35 (17-92)	33 (17-92)	37 (18-127)
Range	5-503	5-503	5-250
Blood blast at best response (%)			
Median (IQR)	0.8 (0.0-18.0)	0.9 (0.0-21.0)	0.0 (0.0-7.0)
Range	0.0-95.0	0.0-95.0	0.0-85.0
BM blast at best response (%)			
Median (IQR)	3.0 (1.0-21.5)	3.7 (1.0-33.0)	3.9 (1.0-32.5)
Range	0.0-96.0	0.0-96.0	0.0-71.0

Mido, midostaurin; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; PR, partial remission; MLFS, morphological leukemia-free state; ORR, overall response rate; NA, not available; WBC, white blood cells; ANC, absolute neutrophil count; Hb, hemoglobin; Pt, platelet; BM, bone marrow; IQR, interquartile range.

^a Composite complete remission (CRC) = CR + CRi + CRp

^b Overall response rate (ORR) = CRC + PR

^c Failure = progression + stable disease

^d Missing data for 11/167, 10/140 and 5/67 patients respectively

Table 5. Gilteritinib-related adverse events during treatment

	Overall study n = 167 (100%) Single agent n=140 Combination n=27	Single agent gilteritinib n = 140 (100%) No prior mido n=70 Prior mido n=70	Single agent gilteritinib and prior exposure to “3+7+mido” n = 67 (100%)
QTcF prolongation			
None	143 (91.7)	120 (92.3)	54 (88.5)
< 450 ms	9 (5.8)	6 (6.2)	6 (9.8)
450-500 ms	4 (2.6)	2 (1.5)	1 (1.6)
> 500 ms	0 (0.0)	0 (0.0)	0 (0.0)
Grade ≥ 3 hematological AE: n (%)			
Anemia	68 (41.0)	56 (40.3)	24 (35.8)
Thrombocytopenia	88 (53.3)	71 (51.4)	37 (56.1)
Neutropenia	87 (52.4)	68 (48.9)	34 (51.5)
Grade ≥ 3 non-hematological AE: n (%)			
Hemorrhage	10 (6.0)	7 (5.0)	5 (7.5)
Hepatic toxicity	7 (4.2)	5 (3.6)	3 (4.5)
Tumor lysis syndrome	1 (0.6)	1 (0.7)	1 (1.5)
Differentiation syndrome: n (%)	10 (6.0)	6 (4.3)	4 (6.0)

Mido, midostaurin; QTcF, Q wave interval (QT)/Fridericia-corrected Q wave; AE, adverse event; HMA, hypomethylating agent; CR, complete remission; RCi, complete remission with incomplete hematologic recovery; IQR, interquartile range.