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

4
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80 Real-world data in R/R *FLT3*-mutated AML treated by gilteritinib

81

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85 **Abstract**

86

87 The real-world efficacy and safety of gilteritinib was assessed in an ambispective study that included
88 167 R/R *FLT3*-mutated AML patients. Among them, 140 received gilteritinib as single agent (cohort B),
89 including 67 previously treated by intensive chemotherapy and midostaurin (cohort C). The main
90 differences in patient characteristics in this study compared to the ADMIRAL trial were ECOG \geq 2
91 (83.6% vs 16.6%), *FLT3*-TKD mutation (21.0% vs 8.5%), primary induction failure (15.0% vs 40.0%) and
92 line of treatment (beyond 2nd in 37.1% vs 0.0%). The rates of composite complete remission, excluding
93 those that occurred after hematopoietic stem cell transplantation (HSCT), were similar at respectively
94 25.4% and 27.5% in cohorts B and C. Median overall survival (OS) for these two groups was also similar
95 at respectively 6.4 and 7.8 months. Multivariate analyses for prognostic factors associated with OS
96 identified female gender (HR 1.61), adverse cytogenetic risk (HR 2.52) and allogenic HSCT after
97 gilteritinib (HR 0.13). Although these patients were more heavily pretreated, these real-world data
98 reproduce the results of ADMIRAL and provide new insights into the course of patients previously
99 treated by intensive chemotherapy and midostaurin and beyond the 2nd line of treatment who can
100 benefit from treatment in an outpatient setting.

101 Introduction

102

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

127 hematologic recovery was 26.3% in the gilteritinib group vs 15.3% in the chemotherapy group⁸. These
128 results led to the approval of gilteritinib monotherapy in US and EU in patients with R/R *FLT3*-mutated
129 AML and it is now a standard treatment in such situations. Nevertheless, in the ADMIRAL trial, only
130 5.7% of patients had been exposed to midostaurin as front line prior to gilteritinib⁹. Therefore, this
131 pivotal trial underrepresented current patients previously exposed to midostaurin, since virtually all
132 *FLT3*-mutated AML patients now receive midostaurin added to standard intensive chemotherapy as
133 first-line therapy⁹.

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

138 **Methods**

139

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

156 **Results**

157

158 **Study population at diagnosis and front-line treatment**

159

160 A total of 167 patients (Cohort A) with *FLT3*-ITD and/or TKD mutated AML fulfilled the inclusion
161 criteria. Among these 167 patients, 27 received gilteritinib associated with another drug and 140
162 received gilteritinib as single agent (Cohort B). Finally, among these 140 patients, 67 received
163 gilteritinib in an R/R setting following a front-line strategy including intensive chemotherapy and
164 midostaurin (Cohort C) (Supplementary Figure 1). Characteristics of patients in these three cohorts are
165 described in Table 1. In cohort A, 73 (43.7%) patients were 65 years of age or older. The median white
166 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
167 *de novo* AML. Cohort A displayed an *FLT3*-ITD mutation in 104 (62.3%) patients and an *FLT3*-TKD
168 mutation in 33 (19.8%), whereas 36 (21.6%) were *FLT3* wild type at diagnosis and acquired mutation
169 later in the disease evolution. Finally, 6 (3.6%) patients had both the *FLT3*-ITD and TKD mutation at
170 diagnosis. Median *FLT3*-ITD/wt ratio was 0.50 (IQR, 0.12-0.70 and range 0.01-1.90), and median size of
171 ITD was 48 bp (IQR, 30-75 and range 3-408). Most patients (136, 81.4%) had an intermediate
172 cytogenetic risk, and 84 (50.3%) had an *NPM1* co-mutation.

173 In cohort A, 153 (91.6%) patients received front-line treatment by intensive chemotherapy, 54
174 (35.3%) a daunorubicin-based “3+7” and 86 (56.2%) an idarubicin-based “3+7”. This intensive
175 chemotherapy was associated with midostaurin in 87 (56.9%) patients, whereas 3 (2.0%) other
176 patients received quizartinib or ponatinib in clinical trials. Median duration of targeted therapy added
177 to intensive chemotherapy was 120.5 days (IQR, 65.0-287.0 and range 9.0-456.0) and 33 patients
178 received maintenance treatment after CR1/CRi1. Among the 153 patients who received an intensive
179 chemotherapy, 125 (81.7%) were in CR1/CRi1 after 1 or 2 courses of intensive chemotherapy.
180 Furthermore, 14 (8.4%) patients received front-line treatment by hypomethylating agents (HMA),
181 including 3 (21.4%) associated with midostaurin. Among the 14 patients who received HMA, 3 (21.4%)

182 were in CR1/CRi1, and median number of cycles of HMA before best response was 5 (IQR, 2.0-6.0 and
183 range 1.0-8.0). Twenty-two (13.2%) patients received an allogenic HSCT in CR1/CRi1. Finally, 128
184 (76.6%) patients in CR1/CRi1 relapsed. The median time to relapse from CR/CRi was 6.6 months (IQR,
185 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
186 received HSCT in CR1/CRi1. Lastly, before gilteritinib, 75 (44.9%) patients received a second-line
187 treatment, 32 (19.2%) patients received a third-line treatment, and 9 (5.4%) patients received a fourth-
188 line treatment. A total of 42 (25.1%) patients received an allogenic HSCT before gilteritinib.
189 Characteristics of patients and results of front-line treatment for cohort B (n=140) and C (n=67) are
190 described in Table 2, with similar results in the three cohorts.

191

192 **Characteristics of patients at gilteritinib initiation**

193

194 The characteristics of the three cohorts at gilteritinib initiation are described in Table 3. In
195 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).
196 Sixty-eight (40.7%) did not have a new karyotypic analysis at relapse, 66 (39.5%) had a karyotype
197 similar to the diagnosis and 27 (16.2%) a different one. Thirty-six (21.5%) patients unmutated at
198 diagnosis acquired an *FLT3* mutation: 27 (75.0%) patients acquired a *FLT3*-ITD mutation and 9 (25.0%)
199 patients a *FLT3*-TKD mutation. Overall, 36 patients among 167 (21.5%) had a single TKD mutation.
200 Finally, at gilteritinib initiation, 25 (15.0%) patients were refractory to front-line treatment, 41 (24.6%)
201 were in 1st relapse \leq 6 months from CR1/CRi1, 39 (23.4%) were in 1st relapse $>$ 6 months from CR/CRi,
202 17 (10.2%) were refractory to the treatment of the 1st relapse and 45 (26.9%) were beyond the 1st
203 relapse. Patients' characteristics were similar in the three cohorts but among 67 patients in cohort C
204 who received gilteritinib as single agent following a front-line strategy including intensive
205 chemotherapy and midostaurin, only 5 (7.5%) were in primary induction failure. Lastly, among 167
206 patients in cohort A, 140 received gilteritinib as single agent (Cohort B) and 27 in combination: 17
207 (10.2%) with an HMA, 6 (3.6%) with venetoclax, and 4 (2.4%) with intensive chemotherapy, low-dose

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

211

212 **Response to treatment**

213

214 Apart from remissions that occurred after HSCT, the response to gilteritinib in the three
215 cohorts is described in Table 4. Among 167 patients in cohort A, the best response to treatment was
216 CR/CRi for 38 (24.4%) patients and CRc (CR+CRi+CRp) for 41 (26.3%). Among 140 patients who received
217 gilteritinib as single agent in cohort B, the best response to treatment was CR/CRi for 30 (23.1%)
218 patients, CRc (CR+CRi+CRp) for 33 (25.4%), overall response rate (ORR) [CRc+ partial response (PR)] for
219 40 (30.8%) patients, and morphological leukemia-free state (MLFS) for 9 (6.9%) patients. Sixty-eight
220 (52.4%) patients were in failure and 6 (4.6%) died before evaluation. In multivariate analysis, no
221 prognostic factor was independently and significantly associated with response among age, ECOG at
222 gilteritinib initiation, secondary AML, sex, white blood cell count (WBC) at gilteritinib initiation,
223 cytogenetic risk, ELN 2017 classification, *FLT3*-ITD, *FLT3*-TKD, ITD/wt ratio, previous HSCT before
224 gilteritinib, prior treatment with midostaurin, and prior treatment with FLT3-TKI. Similar results were
225 observed in cohort C with a best response to treatment that was CR/CRi for 16 (25.9%) patients, CRc
226 for 17 (27.5%) patients, ORR for 21 (34.0%) patients and MLFS for 3 (4.8%) patients. Thirty-one (50.0%)
227 patients were in failure and 3 (4.8%) died before evaluation. Time to the best response was 1.9 months
228 (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.
229 Response to gilteritinib in subgroups defined as per AML phase at gilteritinib initiation are described
230 in Supplementary Table 1. Briefly, among 140 patients who received gilteritinib as single agent, CRc
231 rates were 5.0%, 43.3%, 33.3% and 18.9% in following subgroups : refractory after front-line (N=22),
232 1st relapse ≤ 6 months from CR/CRi (N=32), 1st relapse > 6 months from CR1/CRi1 (N=30) and refractory
233 after 1st relapse and beyond (N=56), respectively. Finally, 32 (19.2%), 25 (17.9%) and 15 (22.4%)

234 patients received an allogenic HSCT after gilteritinib, in CRc for 21 (65.6%), 17 (68.0%) and 11 (73.3%)
235 patients in cohorts A, B and C, respectively (Supplementary Table 2). In cohort A, 12 (37.5%) patients
236 resumed gilteritinib after HSCT as maintenance strategy after a median time from HSCT of 1.6 months
237 (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
238 patients who received maintenance with gilteritinib, only 1 (8.3%) underwent an acute graft versus
239 host disease (GVHD) after 61 days of treatment and 2 (16.7%) a chronic GVHD after 43 and 69 days of
240 treatment.

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

256

257 **Safety**

258

259 Among 98 patients in cohort B with a documented ECG before gilteritinib initiation and who
260 received gilteritinib as single agent, 87 (88.8%) and 11 (11.2%) patients had QTcF <450 ms or >450 but
261 <500 ms, respectively. Finally, 132 (94.3%) patients started gilteritinib at 120 mg per day, 103 (73.6%)
262 did not receive any dose modification, 12 (8.6%) had their posology increased to 200 mg per day, 15
263 (10.7%) had it reduced to 80 mg/day, and 10 (7.1%) had other dose modifications. These modifications
264 were made after a median time of 43 days (IQR 14-72, range 1-368), owing to QTcF modification in 2
265 patients (5.4%), hematological toxicity in 12 (32.4%), lack of efficacy in 13 (35.1%) and other reasons
266 in 10 (27.0%). Reasons for gilteritinib discontinuation were hematological toxicity in 4 (3.0%) patients
267 or other adverse event (AE) in 8 (6.0%) patients, but not for QTcF prolongation. Eighty-nine (66.9%)
268 patients discontinued treatment because of lack of efficacy or death, whereas 18 (13.5%) patients
269 discontinued for HSCT and 14 (10.6%) patients for other reasons. Grade ≥ 3 hematological and non-
270 hematological adverse events are described in Table 5. A total of 6 (4.3%) patients experienced a
271 differentiation syndrome and 1 (0.7%) a tumor lysis syndrome. No posterior reversible encephalopathy
272 syndrome nor acute pancreatitis was noted during the study.

273 Thirty-nine patients (28.3%) experienced a serious AE (SAE) during gilteritinib treatment:
274 infections in 22 (56.4%), hemorrhage in 1 (2.6%), cardiovascular in 4 (10.3%), hepatic in 4 (10.3%),
275 differentiation syndrome in 2 (5.2%), and other SAE in 6 (15.4%). In terms of health care resource
276 consumption, median duration of hospitalization during the first 6 months of treatment was 9.0 days
277 (IQR 0-29, range 0-117) with transfusion of 7 RBC units (IQR 0-14, range 0-54) and 5 platelet units (IQR
278 0-13.5, range 0-56).

279

280 **Outcomes**

281

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

285 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)
286 (Figure 1B), respectively. Moreover, 12-month RFS, EFS and OS were 37.3% (95%CI 18.2-56.5), 23.6%
287 (95%CI 16.5-31.5) and 31.4% (95%CI 22.4-39.7), respectively. Multivariate analyses for factors
288 significantly and independently associated with OS found female gender (HR 1.61, 95%CI 1.07-2.42,
289 $p=0.02$), adverse cytogenetic risk (HR 2.52, 95%CI 1.24-5.13, $p=0.01$) (Figure 2A and B) and HSCT after
290 gilteritinib (HR 0.13, 95%CI 0.05-0.37, $p<0.0001$). Among 33 patients in CRc in cohort B, 15 (45.5%)
291 relapsed. Early death \leq day 30 occurred in 7 (5.0%) patients and \leq day 60 in 18 (12.9%). Among 67
292 patients in cohort C who received gilteritinib in an R/R setting following a front-line strategy including
293 intensive chemotherapy and midostaurin, and after a median FU at 14.5 months (IQR, 9.2-17.7),
294 median EFS and OS were 3.9 months (IQR, 2.4–15.7) and 7.8 months (IQR, 3.2-20.8) (Figure 1C),
295 respectively. Moreover, 12-month EFS and OS were 31.0% (95%CI 20.1-42.4) and 38.3% (95%CI 26.0-
296 50.4), respectively. Among 17 CRc patients in cohort C, 7 (41.2%) patients relapsed. Early death \leq day
297 30 and \leq day 60 occurred in 1 (1.5%) and 10 (14.9%) patients, respectively. Median OS and EFS in
298 various subgroups are described in Supplementary Table 8.

299 We also compared outcomes in Cohort C and in patients who received “3+7” in front-line
300 without midostaurin nor other FLT3-TKI (N=56/126) for OS and EFS that are quite similar and are shown
301 in Figure 2C and D. We also compared OS and EFS depending on treatment added to gilteritinib,
302 showing same median OS and EFS (Supplementary Figure 2A and B). Moreover, patients that were
303 previously exposed to FLT3-TKI other than midostaurin in front- or subsequent-line of treatment
304 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) ($p<0.05$)
305 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)
306 ($p=0.11$) (Supplementary Figure 2C and D). Finally, *FLT3* mutation (ITD vs TKD) showed just a trend to
307 better OS and EFS for *FLT3*-ITD patients treated by gilteritinib, even if 1-year OS was at 36.6% (IC95%
308 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
309 and F).

310

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

312

313 Among the 140 patients of cohort B, 33 (25.4%) were in CRc after gilteritinib treatment, and among
314 them, at the time of data analysis, 15 (46.3%) relapsed. Among these 15 patients, 14 have had a new
315 *FLT3*-ITD evaluation and solely 10 (71.4%) still displayed a *FLT3*-ITD mutation with a median *FLT3*-
316 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-
317 45 and range 2-66). One had also a *FLT3*-TKD D835Y mutation. Finally, among the 67 patients of cohort
318 C, 17 (27.5%) were in CRc after gilteritinib treatment, and among them, at the time of data analysis, 7
319 (41.2%) relapsed. Among these 7 patients, 6 have had a new *FLT3*-ITD evaluation and solely 4 (66.6%)
320 still displayed a *FLT3*-ITD mutation with a median *FLT3*-ITD/wt ratio at 0.50 (IQR, 0.29-0.53 and range
321 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
322 mutation.

323 **Discussion**

324

325 In the current study, we described the characteristics and outcomes of R/R *FLT3*-mutated AML
326 patients in order to evaluate the efficacy and safety of gilteritinib in a real-world setting, especially for
327 patients previously treated by intensive chemotherapy and midostaurin. The main differences in
328 patient characteristics in this study vs the ADMIRAL trial were ECOG ≥ 2 (83.6% vs 16.6%), *FLT3*-TKD
329 mutation (21.5% vs 8.5%), primary induction failure (15.0% vs 40.0%) and line of treatment (beyond
330 2nd in 37.1% vs 0.0% since such patients were excluded from ADMIRAL). Broadly, R/R *FLT3*-mutated
331 AML patients included in this study were in poor general condition, less frequently in primary induction
332 failure but much more heavily pretreated.

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

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

349 7.0 months in the whole cohort and 7.8 months in the subgroup of patients who previously received
350 intensive chemotherapy and midostaurin ¹², so the results of this subgroup are reassuring since they
351 are similar to those of other patients. Another recent study included 33 patients from the CHRYSALIS
352 phase 1/2 trial ¹³ who previously received sorafenib and 33 patients from the ADMIRAL phase 2 trial
353 who previously received midostaurin or sorafenib in 42% and 58% cases, respectively. Median OS was
354 7.2 months and CRc rates were quite similar to those of patients who did not receive prior TKI ¹⁴. To
355 date, our study provides the largest cohort of patients treated by gilteritinib as single agent in real life
356 and the largest cohort of patients receiving gilteritinib after a prior front-line treatment by “3+7” and
357 midostaurin, which is currently the gold standard.

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

372 The main limitation of the present study is obviously its ambispective nature. We cannot
373 exclude selection bias, although we included virtually all patients who received gilteritinib in France
374 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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387

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462

463 **Figure Legends**

464

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

467

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

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513 Alberto Santagostino: no competing interest

514 Marie-Christine Béné: no competing interest

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528

529 **Contributions**

530 Conceptualization, PYD ; Methodology, PYD, EB ; Patients care, PYD, ER, SB, MAH, MH, YD, CB, CP, JL,
531 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,
532 AM, AS, AP, HD, CR ; Collected the data, AM, MCB ; Analyzed data, PYD, EB ; Writing—original draft,
533 PYD ; Revised the manuscript, CR, JL, PP, MH, CO, RG ; Review and editing, PYD, EB, CR ; Funding
534 acquisition, PYD, AM.

535

536 **Data availability**

537 Some de-identified data will be shared with other researchers upon reasonable request to the
538 corresponding authors (pierre-yves.dumas@u-bordeaux.fr). The sharing will require a detailed
539 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 (× 10⁹/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 (× 10⁹/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 (× 10⁹/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.