

## Supplementary Method

All data summarization and analyses were performed using Stata software (Statistical Software: Release 17.0. Stata Corporation, College Station, Texas, USA). Before any analysis, a verification phase of missing, aberrant or inconsistent data took place. Thereafter, the updated database was locked and analyses were carried out on this locked database. Continuous data were summarized descriptively including the number of patients (n), mean, standard deviation, median, interquartile range (IQR = p25-p75), minimum and maximum (on the data provided). Categorical data were summarized by frequencies and percentages. Percentages by categories were based on the number of patients with no missing data, *i.e.* the percentages for the non-missing categories will add up to 100%. Time-to-event data (RFS, OS, EFS) were described by Kaplan-Meier survival analysis with the median (and the IQR) and survival rates at 3, 6, 9, 12 and 24 months. The results were rounded to the nearest tenth. The median follow-up (FU) and its IQR were described by the reverse Kaplan-Meier technique. Prognostic factors of OS (age at initiation of gilteritinib, ECOG at initiation of gilteritinib, *de novo* vs secondary AML, sex, white blood cells at initiation of gilteritinib, cytogenetic risk, ELN 2017 genetic risk, type of mutation of *FLT3*, ITD/wt ratio, prior HSCT, previous treatment with midostaurin, previous treatment with another FLT3-TKI, HSCT post-gilteritinib) were first analyzed by univariate analysis using the HR and its 95% confidence interval (CI) derived from a univariate Cox model. If the sample size allowed, the analysis of the independent prognostic factors on OS was then based, in a second step, on a multivariate Cox model. Covariates shown to be statistically significant (at the threshold of <0.05) in the univariate analysis were then included in the multivariate Cox proportional hazards model. The final model was calculated using a stepwise selection method including only independently statistically significant variables (at the threshold of <0.001). The stricter stay criterion of  $p < 0.001$  is intended for a more parsimonious final model. Intermediate nested models were compared using the likelihood ratio test. The interactions between the independent variables of the final model were evaluated. As recommended, in the event of a significant interaction, the analyses

(Cox-model) were stratified [but no interaction was significant]. The significance level retained was 5% ( $p < 0.05$ ). The two assumptions (log-linearity and proportional risks) of the Cox model were assessed and verified. Predictive response factors were analyzed by percentage comparison tests (chi-square test or Fisher's exact test according to theoretical numbers) for categorical variables and by comparison tests of means (Student's test, if normality and equality of variances) or non-parametric equivalents (Mann-Whitney test) for continuous variables. If the sample size allowed, the variables significantly associated with the response rate in bivariate analysis (at the threshold of  $< 0.05$ ) were introduced into a logistic regression model. The final model comprising the variables significantly (at the threshold of  $< 0.001$  for a more parsimonious final model) and independently associated with the response rate was obtained by the stepwise descending method. Intermediate nested models were compared using the likelihood ratio test. The interactions between the independent variables of the final model were evaluated. As recommended, in the event of a significant interaction, the analyses (logistic regression model) were stratified [but no interaction was significant]. The significance level retained was 5% ( $p < 0.05$ ). The suitability of the model to the data was tested.

**Supplementary Table 1. Response in subgroups among 140 patients who received gilteritinib as single agent**

	<b>Refractory after front-line</b>	<b>1<sup>st</sup> relapse ≤ 6 months from CR/CRi1</b>	<b>1<sup>st</sup> relapse &gt; 6 months from CR/CRi1</b>	<b>Refractory after 1<sup>st</sup> relapse and beyond</b>
	<b>N=22</b>	<b>N=32</b>	<b>N=30</b>	<b>N=56</b>
<b>Best response: n (%)</b>				
CR	0 (0.0)	9 (30.0)	7 (25.9)	6 (11.3)
CRi	1 (5.0)	3 (10.0)	1 (3.7)	3 (5.7)
CRp	0 (0.0)	1 (3.3)	1 (3.7)	1 (1.9)
PR	2 (10.0)	1 (3.3)	3 (11.1)	1 (1.9)
<b>Combined criteria for best response: n (%)</b>				
CR/CRi	1 (5.0)	12 (40.0)	8 (29.6)	9 (17.0)
CRc <sup>a</sup>	1 (5.0)	13 (43.3)	9 (33.3)	10 (18.9)
ORR <sup>b</sup>	3 (15.0)	14 (46.6)	12 (44.4)	11 (20.8)
MLFS	0 (0.0)	1 (3.3)	4 (14.8)	4 (7.5)
Failure <sup>c</sup>	15 (75.0)	12 (40.0)	10 (37.0)	31 (58.5)
No evaluation <sup>d</sup>	1 (5.0)	1 (3.3)	1 (3.7)	4 (7.5)
Deceased before evaluation	1 (5.0)	2 (6.7)	0 (0.0)	3 (5.7)

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.

<sup>a</sup> Composite complete remission (CRc) = CR + CRi + CRp

<sup>b</sup> Overall response rate (ORR) = CRc + PR

<sup>c</sup> Failure = progression + stable disease

<sup>d</sup> Missing data for 2/22, 2/32, 3/30 and 3/56 patients respectively

**Supplementary Table 2. Patients who received HSCT after gilteritinib treatment for R/R situation**

	<b>Overall study n = 167 (100%)</b> Single agent n=140 Combination n=27	<b>Single agent gilteritinib n = 140 (100%)</b> No prior mido n=70 Prior mido n=70	<b>Single agent gilteritinib and prior exposure to "3+7+mido" n = 67 (100%)</b>
<b>HSCT after gilteritinib: n (%)</b>			
Yes	32 (19.2)	25 (17.9)	15 (22.4)
<b>Response at HSCT after gilteritinib: n (%)</b>			
CR	16 (50.0)	12 (48.0)	8 (53.3)
CRi	4 (12.5)	4 (16.0)	2 (13.3)
CRp	1 (3.1)	1 (4.0)	1 (6.7)
PR	2 (6.3)	2 (8.0)	1 (6.7)
MLFS	4 (12.5)	2 (8.0)	0 (0.0)
Failure	3 (9.4)	2 (8.0)	1 (6.7)
No evaluation	2 (6.3)	2 (8.0)	2 (3.3)

Mido, midostaurin; HSCT, allogenic hematopoietic stem cell transplantation; 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; IQR, interquartile range.

**Supplementary Table 3. Baseline characteristics at AML diagnosis for 56 patients treated by single agent gilteritinib and prior exposure to “3+7” without TKI**

	<b>Single agent gilteritinib and prior exposure to “3+7” without TKI n = 56 (100%)</b>
<b>Age (years) at diagnosis</b>	
Median (IQR)	63.8 (48.5-67.9)
Range	20.8-77.9
<b>Gender: n (%)</b>	
Male	31 (55.4)
Female	25 (44.6)
<b>ECOG at diagnosis: n (%)</b>	
0-1	13 (25.5)
≥ 2	38 (74.5)
<b>WBC at diagnosis (× 10<sup>9</sup>/L)</b>	
Median (IQR)	12.7 (5.4-55.4)
Range	1.3-294.0
<b>Blood blast at diagnosis (%)</b>	
Median (IQR)	41.0 (10.0-73.0)
Range	0.0-92.0
<b>BM blast at diagnosis (%)</b>	
Median (IQR)	72.3 (46.0-85.0)
Range	12.0-98.0
<b>AML status: n (%)</b>	
<i>De novo</i>	52 (92.9)
Secondary AML <sup>a</sup>	4 (7.1)
<b>Cytogenetics risk: n (%)<sup>b</sup></b>	
Favorable	2 (3.6)
Intermediate	51 (91.1)
Adverse	1 (1.8)
Failure	2 (3.6)
<b>ELN 2017 prognosis: n (%)</b>	
Favorable	12 (21.4)
Intermediate	37 (66.1)
Adverse	4 (7.1)
Unknown	3 (5.4)
<b>FLT3 mutation: n (%)<sup>c</sup></b>	
FLT3-ITD	24 (42.9)
FLT3-TKD	9 (16.1)
Both	1 (1.8)
No mutation	24 (42.9)
<b>FLT3 ratio ITD/wt: n (%)</b>	
< 3%	2 (9.5)
3–25%	4 (19.0)
26%–50%	5 (23.8)
> 50%	10 (47.6)
<b>NPM1: n (%)</b>	
Mutation	30 (53.6)
No mutation	24 (42.9)

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.

<sup>a</sup> non-*de novo* AML (2 post MDS, 1 post CMML and 1 therapy-related)

<sup>b</sup> according to MRC 2010 classification

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

**Supplementary Table 4. Front-line treatment and relapses for 56 patients treated by single agent gilteritinib and prior exposure to “3+7” without TKI**

	<b>Single agent gilteritinib and prior exposure to “3+7” without TKI n = 56 (100%)</b>
<b>Front-line treatment: n (%)</b>	
Intensive chemotherapy	56 (100.0)
Hypomethylating agent	-
<b>Type of ICT: n (%)</b>	
Daunorubicin-based 3+7	23 (41.1)
Idarubicin-based 3+7	25 (44.6)
GO-based 3+7	4 (7.1)
CPX-351	1 (1.8)
Other	3 (5.4)
<b>FLT3-TKI added to ICT: n (%)</b>	
None	56 (100.0)
Midostaurin	-
Other	-
<b>FLT3-TKI added to HMA: n (%)</b>	
None	-
Midostaurin	-
Other	-
<b>Response to front-line ICT: n (%)</b>	
CR/CRi in 1 course	44 (78.6)
CR/CRi in 2 courses	6 (10.7)
Failure	6 (10.7)
<b>Response to front-line HMA: n (%)</b>	
CR/CRi	-
Partial response	-
Stable disease	-
Failure	-
<b>Relapses after front-line treatment: n (%)</b>	
	50 (89.3)
Bone marrow	43 (86.0)
MRD	6 (12.0)
Extramedullary	1 (2.0)

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.



**Supplementary Table 5. Characteristics at gilteritinib initiation of 56 patients treated by single agent gilteritinib and prior exposure to “3+7” without TKI**

	<b>Single agent gilteritinib and prior exposure to “3+7” without TKI n = 56 (100%)</b>
<b>Age (years) at gilteritinib initiation:</b>	
Median (IQR)	65.4 (49.9-70.4)
Range	23.1-80.4
<b>ECOG at gilteritinib initiation: n (%)</b>	
0-1	9 (16.7)
≥ 2	45 (83.3)
<b>WBC at gilteritinib initiation (× 10<sup>9</sup>/L)</b>	
Median (IQR)	3.3 (1.8-8.0)
Range	0.2-69.0
<b>ANC at gilteritinib initiation (× 10<sup>9</sup>/L)</b>	
Median (IQR)	1.1 (0.4-3.5)
Range	0.0-22.0
<b>Hb at gilteritinib initiation (g/dL)</b>	
Median (IQR)	9.1 (8.4-10.6)
Range	5.5-14.0
<b>Pt at gilteritinib initiation (× 10<sup>9</sup>/L)</b>	
Median (IQR)	35 (16-81)
Range	1-551
<b>Blood blast at gilteritinib initiation (%)</b>	
Median (IQR)	14.1 (1.0-48.0)
Range	0.0-99.0
<b>BM blast at gilteritinib initiation (%)</b>	
Median (IQR)	41.5 (8.0-76.0)
Range	0.0-94.0
<b>AML phase at gilteritinib initiation: n (%)</b>	
Refractory after front-line treatment	6 (10.7)
1 <sup>st</sup> relapse ≤ 6 months from CR/CRi1	9 (16.1)
1 <sup>st</sup> relapse > 6 months from CR/CRi1	9 (16.1)
Refractory after 1 <sup>st</sup> relapse	9 (16.1)
Beyond the 1 <sup>st</sup> relapse	23 (41.1)
<b>Treatment associated with gilteritinib</b>	
None	56 (100)
Intensive chemotherapy	-
HMA	-
LDAC	-
Venetoclax	-
Other	-

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

**Supplementary Table 6. Response to gilteritinib for 56 patients treated by single agent gilteritinib and prior exposure to “3+7” without TKI**

	<b>Single agent gilteritinib and prior exposure to “3+7” without TKI n = 56 (100%)</b>
<b>Best response: n (%)</b>	
CR	9 (17.0)
CRi	4 (7.5)
CRp	2 (3.8)
PR	1 (1.9)
<b>Combined criteria for best response: n (%)</b>	
CR/CRi	13 (24.5)
CRC <sup>a</sup>	15 (28.3)
ORR <sup>b</sup>	16 (30.2)
MLFS	6 (11.3)
Failure <sup>c</sup>	27 (48.2)
No evaluation <sup>d</sup>	2 (3.8)
Deceased before evaluation	2 (3.8)
<b>WBC at best response (<math>\times 10^9/L</math>)</b>	
Median (IQR)	2.7 (1.3-4.3)
Range	0.1-47.2
<b>ANC at best response (<math>\times 10^9/L</math>)</b>	
Median (IQR)	0.7 (0.2-2.0)
Range	0.0-23.0
<b>Hb at best response (g/dL)</b>	
Median (IQR)	10.0 (8.8-11.0)
Range	6.9-14.1
<b>Pt at best response (<math>\times 10^9/L</math>)</b>	
Median (IQR)	36 (24-87)
Range	5-503
<b>Blood blast at best response (%)</b>	
Median (IQR)	0.3 (0.0-22.5)
Range	0.0-95.0
<b>BM blast at best response (%)</b>	
Median (IQR)	3.0 (0.0-33.0)
Range	0.0-96.0

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.

<sup>a</sup> Composite complete remission (CRC) = CR + CRi + CRp

<sup>b</sup> Overall response rate (ORR) = CRC + PR

<sup>c</sup> Failure = progression + stable disease

<sup>d</sup> Missing data for 3/56 patients

**Supplementary Table 7. Composite complete response and overall response rate in various subgroups of patients**

	<b>CRc<sup>a</sup></b> <b>n (%)</b>	<b>ORR<sup>b</sup></b> <b>n (%)</b>	<b>p</b>
<b>Among single agent gilteritinib Cohort B (n=140)</b>			
Previous "3+7" and Mido (n=67)	17 (27.5)	21 (34.0)	0.68
Previous "3+7" without FLT3-TKI (n=56)	15 (28.3)	16 (30.2)	
Adverse cytogenetic (n=9)	0 (0.0)	1 (12.5)	0.04
Favorable/intermediate cytogenetic (n=131)	33 (27.1)	39 (32.0)	
Previous front- or subsequent-line with Mido (n=64)	18 (30.5)	22 (37.3)	0.91
Previous front- or subsequent-line with other FLT3-TKI (n=25)	6 (25.0)	7 (29.2)	
<i>FLT3</i> -ITD positive and <i>FLT3</i> -TKD negative (n=100)	27 (29.4)	34 (37.0)	0.33
<i>FLT3</i> -ITD negative and <i>FLT3</i> -TKD positive (n=28)	4 (15.4)	4 (15.4)	
<b>Among the whole Cohort A (n=167)</b>			
Gilteritinib single agent cohort A (n=140)	33 (25.4)	40 (30.8)	0.08
Gilteritinib associated with other(s) drug(s) (n=27)	8 (30.8)	11 (42.3)	

Mido, midostaurin; TKI, tyrosine kinase inhibitor

<sup>a</sup> Composite complete remission (CRc) = CR + CRi + CRp

<sup>b</sup> Overall response rate (ORR) = CRc + PR

**Supplementary Table 8. Median OS and EFS in subgroups**

	<b>Overall study n = 167 (100%)</b> Single agent n=140 Combination n=27	<b>Single agent gilteritinib n = 140 (100%)</b> No prior mido n=70 Prior mido n=70	<b>Single agent gilteritinib and prior exposure to "3+7+mido" n = 67 (100%)</b>
<b>Overall survival (months) Median (IQR)</b>			
Total	6.4 (3.2-14.3)	6.4 (3.2-14.7)	7.8 (3.2-20.8)
ELN favorable	7.1 (2.8-14.3)	7.1 (3.5-14.3)	7.4 (3.8-16.7)
ELN intermediate	6.4 (3.6-16.2)	6.9 (3.6-20.8)	7.9 (3.7-20.8)
ELN adverse	4.8 (2.1-12.3)	2.6 (1.5-10.0)	2.1 (1.4-5.4)
HSCT before gilteritinib	6.3 (2.2-14.3)	6.3 (2.2-14.3)	6.0 (1.8-13.0)
Refractory after 1 <sup>st</sup> line	6.9 (4.5-11.8)	6.9 (4.5-11.8)	7.0 (4.5-11.8)
1 <sup>st</sup> relapse ≤ 6 months after CR1/CRi1	6.2 (2.8-NR)	6.2 (2.2-NR)	8.0 (3.1-NR)
1 <sup>st</sup> relapse > 6 months after CR1/CRi1	11.2 (3.9-NR)	11.7 (3.9-NR)	16.2 (3.6-NR)
Beyond 1 <sup>st</sup> relapse	5.5 (2.6-10.6)	5.5 (2.4-8.6)	4.8 (2.1-8.6)
<b>Event-free survival (months) Median (IQR)</b>			
Total	4.1 (1.8-11.0)	3.9 (1.6-11.3)	3.9 (2.4-15.7)
ELN favorable	6.2 (2.3-13.5)	5.4 (2.6-13.5)	3.2 (2.6-16.7)
ELN intermediate	4.4 (2.1-10.6)	4.0 (2.1-11.3)	4.5 (2.9-12.9)
ELN adverse	2.8 (1.4-4.9)	1.8 (1.3-3.2)	1.4 (1.0-3.1)
HSCT before gilteritinib	4.5 (1.5-11.6)	3.9 (1.5-11.6)	2.4 (1.3-3.9)
Refractory after 1 <sup>st</sup> line	4.4 (2.1-8.1)	4.0 (1.9-8.1)	4.5 (2.1-6.2)
1 <sup>st</sup> relapse ≤ 6 months after CR1/CRi1	4.4 (2.0-NR)	3.2 (1.6-NR)	3.2 (2.4-NR)
1 <sup>st</sup> relapse > 6 months after CR1/CRi1	7.8 (2.9-NR)	6.2 (2.9-NR)	6.0 (2.9-NR)
Beyond 1 <sup>st</sup> relapse	3.2 (1.4-6.4)	3.2 (1.4-6.3)	3.0 (1.3-4.6)

Mido, midostaurin; ELN, European LeukemiaNet; HSCT, hematopoietic stem cell transplantation; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; NR, not reached; IQR, interquartile range.