

Review

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Review

Gilteritinib: The Story of a Proceeding Success into Hard-to-Treat FLT3 Mutated AML Patients

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Abstract: The traditionally dismal outcome of acute myeloid leukemia (AML) patients carrying the FMS-related tyrosine kinase 3 (FLT3) mutations has been mitigated by the recent introduction into the clinics of tyrosine kinase inhibitors (TKI) such as midostaurin and gilteritinib. The present work summarizes the clinical data that led to the use of gilteritinib in clinical practice. Gilteritinib is a 2nd generation TKI with deeper single-agent activity than 1st generation drugs against both FLT3-ITD and TKD mutations, in human studies. Moreover, the phase I/II dose-escalation, dose-expansion Chrysalis trial showed an acceptable safety profile of gilteritinib (diarrhea, elevated aspartate aminotransferase, febrile neutropenia, anemia, thrombocytopenia, sepsis, and pneumonia) and a 49% overall response rate (ORR) in 191 FLT3-mutated relapsed/refractory (R/R) AML patients. In 2019, the pivotal ADMIRAL trial showed that the median overall survival was significantly longer in patients treated with gilteritinib than among those receiving chemotherapy (9.3 vs 5.6 months, respectively) and the ORR to gilteritinib was 67.6%, outperforming the 25.8% for chemotherapy arm and leading to the license for its clinical use by the US Food and Drug Administration. Since then, several real-world experiences confirmed the positive results in the R/R AML setting. Finally, gilteritinib based combinations currently under investigation with several compounds (venetoclax, azacitidine, conventional chemotherapy, etc.) and some practical tips (maintenance after allogeneic transplantation, interaction with antifungal drugs, extramedullary disease, and onset of resistance) will be analyzed in detail in the review.

Keywords: FLT3 mutations; resistant/relapsed acute myeloid leukemia; tyrosine kinase inhibitors; gilteritinib

1. Introduction

It is now widely accepted that the class III receptor tyrosine kinase FLT3 mutation status distinguishes a subtype of acute myeloid leukemia (AML) with a poor prognosis. Indeed, FLT3 mutated AMLs retain higher relapse rates, shorter remission duration following initial therapy (6 months vs. 11.5 months for those without *FLT3* internal tandem duplication [ITD] mutations), as well as reduced disease-free survival (16% to 27% vs. 41% at 5 years) and overall survival (OS) (15% to 31% vs. 42% at 5 years) [1–3]. Relapsed/refractory (R/R) AML has a median OS of 4–7 months with standard chemotherapy approaches [4–7], emphasizing the importance of newly approved targeted therapies and the need for additional treatment options. *FLT3*/ITD and *FLT3*/TKD mutations are ideal targets for small molecule inhibitors. On September 21st, 2018, gilteritinib was approved in Japan for the treatment of R/R *FLT3*-mutated AML; on November 28th of the same year, also the US Food and Drug Administration (FDA) declared marketing approval of gilteritinib for the same indication in the United States. In the phase III ADMIRAL study, gilteritinib considerably outperformed salvage chemotherapy in terms of OS and the response rate for complete remission with full or partial hematological recovery maintaining a manageable toxicity profile [8]. Furthermore, gilteritinib represented a valid treatment approach as bridge to transplant in this critical subgroup of AML [8]. Overall, these data have led to a therapy shift into the AML treatment scenario establishing gilteritinib as the new gold standard for R/R *FLT3*-mutated AML, while incorporation of the drug into frontline regimens will likely become the standard therapeutic strategy for *de novo* *FLT3*-mutated

AML. In the next future, novel combination approaches promise to further revolutionize the therapeutic landscape of this AML setting. This review will discuss clinical trials and real-life studies data of gilteritinib in R/R AML and as maintenance approach after transplant, and will explore alternative combinations with chemotherapy or other small molecules in *de novo* and R/R AML.

2. Pharmacodynamics and pharmacokinetics of gilteritinib

Gilteritinib is a next-generation tyrosine kinase inhibitor (TKI) primarily targeting FLT3 and AXL (an onco-genic tyrosine kinase) receptors [9]. Compared to first-generation multi-targeted TKIs, it is more selective to FLT3 and has greater potency. It blocks FLT3 receptors' ATP-binding site competitively, thus inhibiting receptor signaling and halting cell cycle [10]. Cellular experiments have shown powerful inhibitory effects on FLT3 mutations (FLT3-ITD and FLT3-D835Y point mutations in particular) [11]. Since both FLT3- ITD and FLT3-TKD mutations promote constitutive FLT3 kinase activity, sustaining leukemic cell proliferation and survival, gilteritinib mediated inhibitory effects have the potential to lessen the leukemia burden of AML patients (Figure 1). It is classified as a type I inhibitor, generally unaffected by mutations in the activation loop (e.g., at D835) [12]. Moreover, gilteritinib promotes apoptosis in FLT3-ITD mutations carrying tumor cells *in vitro* [9]. In xenografted mice models, oral administration of gilteritinib lowered phosphorylated FLT3 levels by 40% after 1 hour [11], while a single dosage was sufficient to reduce the phosphorylation of STAT-5, a known downstream FLT3 target [11]. Following successive gilteritinib 120 mg doses in patients with R/R-AML, approximately 90% of FLT3 phosphorylation was decreased, with inhibition starting to take place 24 hours after the first dosage [9]. When oral gilteritinib (1–10 mg/kg) was given to mice once every day for 28 days, tumor development was significantly suppressed by 63–100% ($p=0.05$) [11]. Although gilteritinib did not influence the *in vitro* reduction of tumor growth or induction of apoptosis, stimulation of the FLT3 ligand can raise the chance of resistance to other FLT3 inhibitors [13]. Given that AXL activation is a known resistance mechanism to FLT3 inhibitors and that AXL inhibition can slow the growth of FLT3-ITD AML tumors, gilteritinib additional activity against AXL may also be advantageous [14]. In comparison to other less specific TKIs, gilteritinib may present a lower clinical risk of side events, such as myelosuppression [11]. Inhibition of c-KIT (an oncogene encoding KIT, a platelet-derived growth factor receptor essential for hematopoiesis) is expected to provoke severe myelosuppressive effects because FLT3 and KIT structures are remarkably similar [15]. Thus, the risk of myelosuppression with gilteritinib is anticipated to be lower than with other TKIs because it has no impact on c-KIT [15]. Based on *in vitro* findings, CYP3A4 primarily metabolizes gilteritinib [15]. The main metabolites identified in animal investigations are M17, M16, and M10 (all accounting for less than 10% of the parent exposure); it is unknown if these metabolites have any effect on FLT3 or AXL receptors [9]. Since that gilteritinib is a P-glycoprotein (P-gp) substrate, a multidrug transporter that actively pumps substances out of the cell and away from their target regions [16], it might exert an inhibitory effect on BCRP, P-gp, and OCT1 in the small intestine as well as the liver [9]. *In vivo*, gilteritinib neither induces nor inhibits CYP3A4 or MATE1. Since gilteritinib may decrease the effectiveness of 5-HT2B or sigma non-specific receptor targeting medications *in vitro* (such as escitalopram), it should only be used in rare conditions together with these medications [9]. Reduced gilteritinib plasma concentrations are caused by co-administration with a P-gp and potent CYP3A inducer, hence this should be avoided [9]. Conversely, gilteritinib exposure is increased when it is administered concurrently with a potent CYP3A and/or P-gp inhibitor [15]. For instance, co-administration of a single 10 mg dose of gilteritinib with 200 mg of itraconazole per day for 28 days raised Cmax and AUC in healthy individuals by 20% and 120%, respectively [9]. A concurrent strong CYP3A and/or P-gp inhibitor increased exposure in individuals with R/R-AML by about 1.5 times [9].

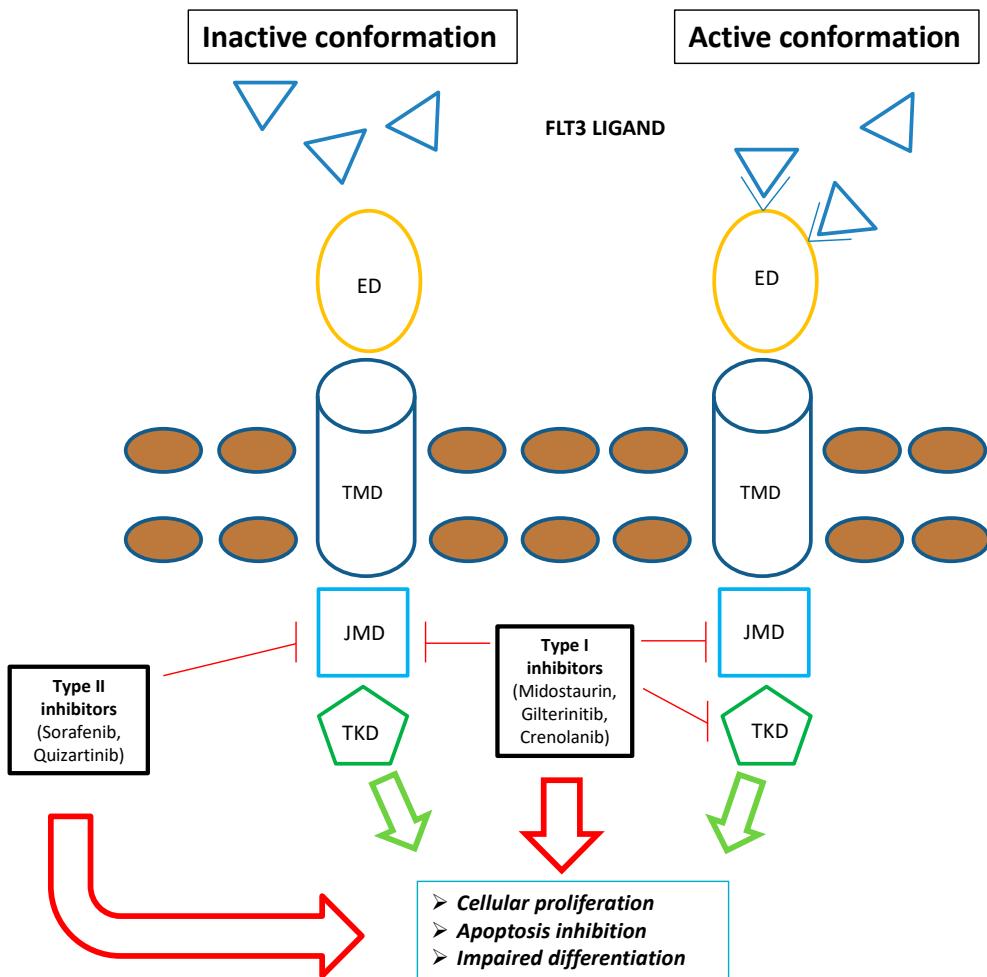


Figure 1. Schematic representation of FLT3 inhibitors mechanism of action.

Type I family of FLT3 inhibitors (Midostaurin, Gilteritinib and Crenolanib) is able to bind the FLT3 receptor both in the active and inactive conformation inhibiting FLT3-ITD and TKD mutations. Contrarywise, type II family of FLT3 inhibitors (Sorafenib and Quizartinib) is able to bind the FLT3 receptor in the inactive conformation acting only on FLT3-ITD. Overall, FLT3 inhibitors severely compromise leukemogenic activity of FLT3 (i.e. cellular proliferation, apoptosis inhibition, impaired differentiation). Green arrows: FLT3 mediated leukemogenic activity in the absence of FLT3 inhibitors; Red Arrows: impairment of FLT3 mediated leukemogenic activity in the presence of FLT3 inhibitors

Abbreviations: FLT3, FMS-like tyrosine kinase; ED, extra-membrane domain; TMD, transmembrane domain; JMD, juxtamembrane domain.

3. Clinical trials including gilteritinib as monotherapy

3.1. Chrysalis trial

Gilteritinib was evaluated for its safety, pharmacokinetics, pharmacodynamics, and anti-leukemic activities in this first-in-human, open label phase I/II dose-escalation, dose-expansion Chrysalis trial (NCT02014558) in patients with R/R AML. This study included patients with wild-type (wt) FLT3 ($n = 58$) and FLT3 mutation ($n = 191$), totaling 252 R/R AML patients. Participants were assigned to receive a once-daily oral dose of gilteritinib ranging from 20 mg to 450 mg and were enrolled in one of seven dose-escalation ($n = 23$) or dose-expansion ($n = 229$) groups. Overall, gilteritinib was well tolerated; the maximum tolerated dose (MTD) was established at 300 mg/day

when two out of three patients enrolled in the 450-mg dose-escalation cohort had two dose-limiting toxicities (grade 3 diarrhea and grade 3 elevated aspartate aminotransferase). Most frequent grade 3–4 adverse events (AEs) included febrile neutropenia (39%), anemia (24%), thrombocytopenia (13%), sepsis (11%), and pneumonia (11%); death occurred in ninety-five patients, with seven deaths judged possibly or probably related to treatment. At least 90% of FLT3 phosphorylation inhibition was observed by day 8 in most patients receiving a daily dose of ≥ 80 mg. Overall Response Rate (ORR) in the entire population was 40%; ORR in FLT3 mutated (n = 191) and FLT3wt (n = 58) patients was 49% and 12%, respectively. Remarkably, the ORR was enhanced in FLT3 mutated patients at doses ≥ 80 mg/day resulting in 52%. The median OS in the two subgroups was 30 and 17 weeks, respectively [17]. In FLT3 mutated patients with R/R AML, gilteritinib monotherapy was well tolerated and produced frequent and persistent clinical responses. In FLT3 mutated patients treated at levels that consistently and potently suppressed FLT3 phosphorylation, anti-leukemic responses were enhanced. Gilteritinib related poor efficacy in patients without FLT3 mutations suggested that this approach is very selective by its activity against the FLT3.

3.2. ADMIRAL trial

The phase III ADMIRAL trial showing improved OS with gilteritinib versus salvage chemotherapy in patients with R/R FLT3-mutated AML has led to the FDA approval of the drug in this setting. 371 patients from 14 different countries have been randomly enrolled 2:1 to receive gilteritinib at 120 mg/die (n=124) or investigator's choice of salvage chemotherapy (MEC, FLAG-IDA, low-dose cytarabine or azacitidine) (n=124) with cycles of 28-days. ITD-FLT3 mutation was detected in 87% and 91% in the gilteritinib and salvage chemotherapy group, respectively and the preselected salvage chemotherapy was a high-intensity regimen in 60% of both groups. After a median follow-up of 17.8 months, the median OS was 9.3 versus 5.6 months ($p < .001$) in the two subgroups, respectively with the benefit of gilteritinib maintained also in analysis-censoring survival data at the time of allogenic hematopoietic stem cell transplantation (HSCT). The rate of complete remission (CR) with full or partial hematologic recovery in the two groups was 34% versus 15.3% (absolute 18.6% risk difference) with a median duration of response in the gilteritinib group of 11 months. The median event-free survival (EFS) was significantly different between the two subcategories (2.8 vs 0.7 months; HR 0.79, 95% CI=0.58-1.09). The incidence of exposure-adjusted grade ≥ 3 AEs was 19.3 and 42.4 events per patient-year in the gilteritinib and salvage chemotherapy, respectively, while the incidence of serious AEs was 7.1 versus 9.2 per patient-year in the two subgroups. Drug-related AEs that led to discontinuation of gilteritinib occurred in 11% of patients; the most common gilteritinib related fatal AEs were pneumonia (1.2%), large intestine perforation (0.8%) and septic shock (0.8%) [8]. Recently, a 2 years' follow-up of the ADMIRAL trial after the primary analysis was reported to clarify the long-term treatment effects and safety of gilteritinib in FLT3 mutated R/R AML. The 2-year estimated survival rates were 20.6% and 14.2% in the gilteritinib and salvage chemotherapy groups; the survival benefit of gilteritinib was maintained in the FLT3-ITD mutation subgroup and in patients with a high FLT3-ITD allelic ratio, while it was not observed in the FLT3-TKD subgroup and in patients with a low FLT3-ITD allelic ratio. The 2-year cumulative relapse rates in gilteritinib-treated patients who achieved a CR or composite CR were 52.6% and 75.7%, respectively. Twenty-six patients treated with gilteritinib were still alive after 2 years of treatment without relapsing; among them, 18 underwent HSCT and 16 received gilteritinib after transplant. In this setting, most patients were aged <65 years (84.6%), treated with high intensity treatment before randomization (76.9%) and had not received previous FLT3 inhibitors (96.1%). The most common reported AEs during the first and second year of treatment were the increased levels of transaminases. Compared with the first year of gilteritinib therapy, in the second year it was observed a reduced incidence of these AEs [18]. These data confirmed the long-term benefit of gilteritinib treatment either in patients who did not undergo to transplant or in patients who continued gilteritinib in the post-transplant phase. Recently, Smith et al. analyzed the molecular profile of R/R AML patients enrolled in ADMIRAL trial focusing on the potential relationship between co-mutations in molecular partners of FLT3 and response to treatment [19]. At the time of enrollment, patients were classified in the following subgroups: DNA

methylation/hydroxymethylation (41.2%), transcription factors/regulators (26.3%), chromatin-spliceosome-other (17.4%), receptor tyrosine kinase (RTK)-Ras signaling (7.8%), TP53-aneuploidy (3.6%), *NPM1* (47.9%), *DNMT3A* (31.9%), *DNMT3A/NPM1* (23.8%), *WT1* (18.0%), and *IDH1/IDH2* (15.5%). Response rates before HSCT appeared higher in the gilteritinib arm versus the standard chemotherapy arm across all gene categories except TP53-aneuploidy, which included a small series of patients (n = 13). Longer survival was identified among *NPM1*-mutated, DNA methylation/hydroxymethylation, and transcription factor categories as well as in co-mutated *DNMT3A*, *WT1*, and dual-mutated *DNMT3A* and *NPM1* gene categories in the gilteritinib arm as compared to the standard chemotherapy arm. Patients with *DNMT3A/NPM1* co-mutations treated with gilteritinib showed the most favorable outcomes compared with all the others molecular subgroups. Furthermore, OS results observed with gilteritinib were not negatively impacted by *FLT3*-ITD allelic ratio, *FLT3*-ITD length, or multiple *FLT3*-ITD mutations. In the subgroup of patients with *FLT3*-ITD lengths >51 bp, the median OS was 10.4 vs. 6.0 months in the gilteritinib arm and in the standard chemotherapy arm (HR = 0.480; 95% CI, 0.311-0.742), while among patients who presented at baseline multiple *FLT3*-ITD mutations, median OS was 8.3 months and 3.5 months, respectively (HR = 0.624; 95% CI, 0.331-1.175). In addition, patients with a high *FLT3*-ITD allelic ratio (≥ 0.77) who received gilteritinib showed a significantly longer OS (7.1 vs 4.3 months; HR = 0.49; 95% CI, 0.34-0.71). Of the 247 gilteritinib treated patients, relapse was observed in 75 patients (30%) who had achieved any type of CR; among them, 40 (53.3%) had blood or bone marrow samples available for analysis at baseline and relapse. Overall, 27 out of the 40 relapsed patients (67.5%) had developed new gene mutations during gilteritinib therapy. New mutations in Ras/MAPK pathway genes were detected in 18 patients at the time of relapse with the most frequently mutated Ras/MAPK pathway genes including *NRAS* (61.1%), *PTPN11* (44.4%), and *KRAS* (38.9%); however, the presence of Ras/MAPK pathway gene mutations at baseline did not affect a potential response to gilteritinib (rate of composite CR before HSCT in gilteritinib-treated patients with Ras/MAPK pathway gene mutations at baseline was 33.3%) [19]. These results shed light on the molecular profile of *FLT3*-mutated R/R AML, the effect of *FLT3* inhibitors on mutational evolution, linked to treatment resistance, and the efficacy of gilteritinib across a broad range of molecular and genetic subgroups.

Perl et al. [20] retrospectively compared clinical outcomes of patients enrolled in the CHRYSLIS and ADMIRAL trials who had received prior midostaurin or sorafenib against those without prior *FLT3* tyrosine kinase inhibitor (TKI) exposure. Patients who received a *FLT3* TKI prior to gilteritinib (CHRYSLIS, 42%; ADMIRAL, 52%) and those who did not (CHRYSLIS, 43%; ADMIRAL, 55%) both showed high rates of composite complete remission (CRc). In ADMIRAL, among patients who had previously received a *FLT3* TKI, the gilteritinib arm had a higher CRc rate (52%) and a tendency toward a longer median OS than the standard chemotherapy arm (CRc = 20%; overall survival, 5.1 months; HR = 0.602; 95% CI: 0.299, 1.210). With prior *FLT3* TKI exposure, the duration of remission was shorter [20]. These results established gilteritinib as a valid treatment option also for patients with *FLT3*-mutated R/R AML who had previously received sorafenib or midostaurin. **Table 1** summarizes the most significant efficacy and outcomes data of ADMIRAL trial.

Table 1. Rate of responses and outcomes in ADMIRAL study.

Response data		
Overall Response in <i>FLT3</i> mutated AML¹ (n=247)		67.7
• ORR ² (%)		
• Complete remission or complete remission with partial hematologic recovery (%)		34
• Complete remission (%)		21.1
• No response (%)		
• Median duration of remission (months)		26.7
		11
Rate of response in <i>FLT3</i> mutated AML previously treated with TKIs³ (n=33)		
• CRc ³ (%)		17

• Median complete response duration (months)	8.9
• CRc ³ (%) in AML who had previous midostaurin (n=14)	57
• CRc ³ (%) in AML who had previous sorafenib (n=19)	
• Median complete response duration (months) in AML who had previous midostaurin	47
• Median complete response duration (months) in AML who had previous sorafenib	3.7
	12.9

Rate of response in FLT3 mutated AML¹ by baseline co-mutations (n=239)	29
• CR ⁴ /CRh ⁵ (%) in patients with DNA methylation/hydroxymethylation (n=100)	17.2
• CR ⁴ /CRh ⁵ (%) in patients with transcription factors/regulators (n=64)	12.8
• CR ⁴ /CRh ⁵ (%) in patients with chromatin–spliceosome–other (n=47)	20
• CR ⁴ /CRh ⁵ (%) in patients with RTK-Ras signaling (n=10)	28.6
• CR ⁴ /CRh ⁵ (%) in patients with TP53-aneuploidy (n=15)	27
• CR ⁴ /CRh ⁵ (%) in patients with NPM1 (n=105)	29.3
• CR ⁴ /CRh ⁵ (%) in patients with DNMT3A (n=75)	30.9
• CR ⁴ /CRh ⁵ (%) in patients with DNMT3A/NPM1 (n=55)	13.3
• CR ⁴ /CRh ⁵ (%) in patients with WT1 (n=45)	
• CR ⁴ /CRh ⁵ (%) in patients with IDH1/IDH2 (n=38)	28.2

Outcomes data

Outcomes of FLT3 mutated AML¹ (n=247)	9.3
• Median OS ⁶ (months)	
• 1-year OS ⁶ (%)	36.6
• 2-year OS ⁶ (%)	20.6
• 3-year OS ⁶ (%)	15.8
• 2-year cumulative relapse rate in patients who achieved a CR ⁴ (%)	52.6
• 2-year cumulative relapse rate in patients who achieved a CRc ³ (%)	75.7

Outcomes of FLT3 mutated AML¹ according to previous TKIs⁷ therapy	9.5
• OS ⁶ duration (months) in patients who did not receive prior TKIs ⁷ (n=33)	8.7

Overall survival in FLT3 mutated AML by baseline co-mutations (n=239)	11.4
• OS ⁶ (months) in patients with DNA methylation/hydroxymethylation (n=100)	9.6
• OS ⁶ (months) in patients with transcription factors/regulators (n=64)	7.1
• OS ⁶ (months) in patients with chromatin–spliceosome–other (n=47)	4.6
• OS ⁶ (months) in patients with RTK-Ras signaling (n=10)	10.6
• OS ⁶ (months) in patients with TP53-aneuploidy (n=15)	8.6
• OS ⁶ (months) in patients with NPM1 (n=105)	11
• OS ⁶ (months) in patients with DNMT3A (n=75)	15.1
• OS ⁶ (months) in patients with DNMT3A/NPM1 (n=55)	8.3
• OS ⁶ (months) in patients with WT1 (n=45)	
• OS ⁶ (months) in patients with IDH1/IDH2 (n=38)	15.4

Outcomes of FLT3 mutated AML¹ according to FLT3-ITD length, multiple FLT3-ITD mutations and

FLT3-ITD allelic ratio	10.4
• OS ⁶ (months) in patients who had FLT3-ITD lengths >51 bp (n=90)	8.9
• OS ⁶ (months) in patients who had FLT3-ITD lengths ≤51 bp (n=99)	

• OS ⁶ (months) in patients with multiple FLT3-ITD mutations at baseline (n=33)	9.3
• OS ⁶ (months) in patients with high (≥ 0.77) FLT3-ITD allelic ratio (n=109)	7.1
• OS ⁶ (months) in patients with low (< 0.77) FLT3-ITD allelic ratio (n=113)	10.6

AML= acute myeloid leukemia. ORR= overall response rate. CRc= composite complete remission. CR= complete remission. CRh= complete remission with partial hematologic recovery. OS= overall survival. TKI= tyrosine kinase inhibitor.

4. Real-life experiences with gilteritinib in R/R AML

The French AML Intergroup ALFA/FILO retrospectively analyzed a real-world series of R/R *FLT3*-mutated AML patients (n=167) treated with gilteritinib as monotherapy. Most patients had received front-line treatment with intensive chemotherapy, with approximately half receiving chemotherapy plus midostaurin (n=67). Composite CR rates (25.4% and 27.5%) and median OS (6.4 and 7.8 months) were similar with prior midostaurin exposure or not, and comparable with those observed in the ADMIRAL trial [8]. However, when compared to the results of the ADMIRAL trial, higher rates of grade ≥ 3 thrombocytopenia, but equal rates of anemia were observed [21]. These findings support the use of gilteritinib, even in intensively treated patients who have received midostaurin as front-line therapy.

In order to reduce the rate of mortality and the utilization of healthcare resources, the United Kingdom National Health Service (NHS) made gilteritinib available as an emergency measure to patients aged > 16 years with R/R *FLT3* mutant AML starting from April 2020. A multi-centric analysis in UK evaluated 50 R/R AML patients treated with gilteritinib; among them, most patients had previously received 1 (65%) or 2 (33%) lines of therapy, including intensive chemotherapy in a majority (86%). 45 % of patients had received a previous TKI inhibitor and 35% had relapsed after HSCT. A previous exposure to *FLT3* inhibitor ($p > 0.9$) and HSCT ($p = 0.3$) did not influence the median OS that was 6.7 months. The composite CR / CR with incomplete hematological recovery (CRi) rate was 27% and the mortality at day 30 and day 60 was 0% and 14%, respectively. Median time of hospitalization was 3.5 days in cycle 1, 0 days in cycles 2 and 3 and 1 day in cycle 4 [22].

The largest US multi-institutional retrospective analysis was recently reported. A total of 113 R/R AML patients was analyzed; most of them received gilteritinib as a single-agent therapy (62.8%), while the rest of patients were treated gilteritinib based combinations [intensive chemotherapy (31%), hypomethylating agents 33%, venetoclax or hypomethylating and venetoclax 31% and IDH inhibitors 5%). 55 (48.7%) patients achieved a CRc with CR in 25 patients (22.1%) the median OS was 7.0 months. A trend toward a higher CRc rate was observed in patients who received gilteritinib with combination treatments rather than as a single agent (64% vs 43%, respectively, $p = .09$); however, no survival benefit was reported for combination therapy compared with single agent approach. The presence at baseline of *NRAS*, *KRAS*, and *PTPN11* mutations which are known to confer gilteritinib resistance was correlated with lower CRc (35% vs. 60.5%) and lower median OS than patients who did not express these mutations (4.9 months vs. 7.8 months; $p < .01$) [23].

Also, the Israeli group retrospectively analyzed 25 patients from six academic centers who received gilteritinib for *FLT3*-mutated R/R AML; most of them (80%) was treated with prior intensive chemotherapy and almost half (40%) with TKI therapy. The rate of CR was 48% with an estimated OS of 8 months. Prior TKI exposure did not negatively impact on OS and was associated with superior EFS ($p = .016$). The authors performed an age and ELN-risk matched comparison between patients who received gilteritinib and intensive salvage treatments. This analysis showed similar response rates (50% in both groups) and median OS (9.6 months vs 7 months; $p = 0.869$) in the two groups, respectively [24]. Altogether, these studies showed comparable efficacy of gilteritinib in the real-life setting to the pivotal ADMIRAL trial (Table 2).

Table 2. Real life studies including gilteritinib as monotherapy in relapsed/refractory acute myeloid leukemia.

Reference	Number of patients	Composite		Median overall survival	Comment
		complete	remission		
Dumas et al. ²¹	140 (cohort B) 67 previously treated by intensive chemotherapy and midostaurin (cohort C)	25.4% (cohort B) 27.5% (cohort C)		6.4 months (cohort B) 7.8 months (cohort C)	prognostic factors associated with OS identified female gender (HR 1.61), adverse cytogenetic risk (HR 2.52), and allogenic transplant after gilteritinib (HR 0.13)
Othman et al. ²²	50 (86% received previous intensive chemotherapy)	27%		6.7 months (95%CI 4.5 - not reached)	the rate of composite complete response did not differ in those with previous exposure to FLT3 inhibitors (23% vs 32%, p=0.6) or with past allogeneic transplant (29% vs 27%, p=0.3)
Numan et al. ²³	113 (62.8% received gilteritinib as monotherapy, while the remaining patients received gilteritinib in combination with other agents)	48.7%	group 7.1 months for none-transplant 7.8 months in patients treated with prior midostaurin 5 months in patients treated with prior sorafenib	7.4 months for transplant	The presence of PTPN11 and NRAS had a significant inferior impact on composite complete remission rate (59% vs. 37.5%) and median overall survival (4.9 months vs. 7.8 months; HR 2.4– 95% CI 1.1–5.4 -p = .0057)
Shimony et al. ²⁴	25 (80% treated with prior intensive chemotherapy and 40% previously treated with tyrosine kinase inhibitor therapy)	48%		8 months	Prior tyrosine kinase inhibitor exposure did not negatively impact on overall survival and was associated with superior event-free survival (p = 0.016)

5. Safety Profile of gilteritinib

In patients with R/R FLT3-mutated AML, gilteritinib exhibited an overall good safety profile. The integrated safety population (results from a phase I trial in Japanese patients [14], the phase I/II Chrysalis [17] and phase III ADMIRAL [8] studies) who received ≥ 1 dose of gilteritinib 120 mg (n = 319), is the main issue of this section. These patients were exposed to gilteritinib for an average time of 3.6 months. 83.1% of patients experienced a treatment-related AE (TRAE) [9]. Anemia, febrile neutropenia, and thrombocytopenia were the most frequent grade 3 TRAEs observed in 60.2% of patients. The 33.9% of patients had serious TRAEs; the most common were febrile neutropenia, elevated alanine aminotransferase (ALT), and elevated aspartate aminotransferase (AST) levels [9]. Six percent and 29% of patients who received gilteritinib experienced dose reduction or stoppage due to an AE, respectively, while 7% of patients discontinued treatment due to an AE. Increased

transaminases (51% of patients), myalgia or arthralgia (50%), fatigue or malaise (44%), fever (41%), mucositis (41%), oedema (40%), rash (36%), non-infectious diarrhea (35%), dyspnea (35%) and nausea (30%) were the most common non-hematological AEs of any grade (incidence 30%) [9]. The most frequent serious non-hematological AEs (incidence 5%) were: fever (13%), dyspnea (9%), renal impairment (8%), elevated transaminases (6%) and non-infectious diarrhea (5%). Among 2% of those who received gilteritinib, there were fatal AEs: cardiac arrest (1%), differentiation syndrome (1%), and pancreatitis (1% each) [9]. Although rarely occurring, gilteritinib treatment resulted in a number of clinically severe AEs of particular interest (AESIs) [9]. In the integrated safety population, differentiation syndrome appeared in 11 patients (3%) between days 2 and 75 after the start of treatment, regardless of the presence of leukocytosis. Most patients recovered after drug interruption. Grade 3 treatment-emergent posterior reversible encephalopathy condition (PRES) was observed in two patients (0.6%). Treatment-related QT prolongation was noted in 7.2% of patients, with 1.9% of those patients having significant QT prolongation. In 1.3% of patients, cardiac failure was deemed grade 3 and treatment-related (it was severe in 0.9% of patients). 2.5% of patients developed a grade 3 treatment-related hypersensitivity responses, and 1.6% of those events were severe (inclusive of one patient who experienced anaphylaxis) (EMA). With the exception of elevated liver transaminases levels, which occurred more frequently in gilteritinib recipients, gilteritinib therapy and salvage chemotherapy in the ADMIRAL trial caused similar TEAEs in the first 30 days of treatment [18]. Except for cough (0.09 vs 0.05 events per patient-year), increased AST level (1.26 vs 0.76 events per patient-year) and increased ALT level (1.22 vs 0.84 events per patient-year), the incidence of all exposure-adjusted TRAEs was lower in gilteritinib receivers than in salvage chemotherapy recipients. Receivers of gilteritinib experienced a frequency of 19.34 events per patient-year and those receiving salvage chemotherapy experienced 42.44 events per patient-year of exposure-adjusted grade 3 TRAEs [17]. Gilteritinib had a stable safety profile beyond 2 years [18]. Real-life studies [21–24] have shown toxicities similar to those of clinical trials, further demonstrating the manageable toxicity profile of gilteritinib.

6. Combination regimens including gilteritinib in R/R and de novo AML

6.1. Gilteritinib plus azacitidine in FLT3-mutated AML

Wang et al. [25] proposed a randomized phase 3 trial aimed to assess the efficacy and safety of gilteritinib plus azacitidine versus azacitidine in newly diagnosed FLT3 mutated AML considered not eligible for intensive chemotherapy. Patients were randomized (2:1) to be treated with gilteritinib (120 mg/day orally) and azacitidine at standard dosage or azacitidine alone, on a 28-days cycle. One-hundred twenty-three patients were enrolled, 74 included in the gilteritinib-azacitidine arm (median age, 78 years) and 49 in the azacitidine arm (median age 76 years); among them, 47.3% and 32.7% had an ECOG performance status (PS) of 2 in the two arms, respectively.

Authors showed no significant difference in OS between the two arms; the median OS was 9.82 months and 8.87 months, respectively (HR 0.916; 95% CI, 0.529-1.585; $p = .753$). The median EFS was 0.03 months in both treatment arms; the CRc rate was significantly higher in the gilteritinib-azacitidine arm than in the azacitidine arm (58.1% and 26.5%, respectively; $p < .001$). Furthermore, authors observed a numeric improvement in OS with gilteritinib-azacitidine in some patient subgroups, but statistical significance was not reached. In the subgroup of patients stratified as having an ECOG PS of 0 to 1, the median OS was 13.17 months and 11.89 months, respectively (HR, 0.811; 95% CI, 0.409-1.608; $p = .549$); among patients with a FLT3-ITD allelic ratio of 0.5 or higher, the median OS was 10.68 months and 4.34 months, respectively (HR, 0.580; 95% CI, 0.285-1.182; $p = .134$). AEs rates were similar between the arms. AEs of any grade occurred in 100% of patients in the gilteritinib-azacitidine arm and 95.7% of those in the azacitidine arm. The rate of grade 3 or higher AEs was 95.9% and 89.4%, respectively [25]. According to these data, this combination approach did not improve survival outcomes in patients with newly diagnosed, FLT3 mutated AML considered unfit for intensive treatment. Therefore, the trial was closed based on the protocol-specified boundary for futility and recommendations from the independent data monitoring committee.

6.2. Gilteritinib plus venetoclax in R/R AML

Venetoclax has been approved as a standard treatment in combination with low-dose cytarabine or hypomethylating agents for newly diagnosed AML ineligible for intensive chemotherapy [26, 27]. Single-agent venetoclax showed limited activity in R/R AML [28]; however, *in vitro* reports demonstrated synergistic activity between venetoclax and FLT3 inhibitors in preclinical models [29, 30].

In a U.S., multicenter study, 61 patients with R/R AML, including 56 with FLT3 mutated disease, were enrolled to receive the combination regimen based on venetoclax and gilteritinib; 15 patients were enrolled in the dose-escalation phase and 46 were enrolled in the dose-expansion phase. The trial provided 400 mg of venetoclax once daily and gilteritinib at 80 mg or 120 mg once daily during dose escalation, with the recommended phase II dose being venetoclax at 400 mg and gilteritinib at 120 mg. Among the 56 patients with FLT3 mutant disease treated at any dose, after a median follow-up of 17.5 months, the modified composite CR (consisting of complete response, complete response with incomplete blood count recovery, complete response with incomplete platelet recovery, and morphologic leukemia-free state) rate was 75% (the CR rate was 18%). Median time to response and median remission duration was 0.9 months and 4.9 months, respectively with a median OS of 10.0 months. Modified composite CR was observed in 14 (67%, CR in 29%) of 21 patients with no prior FLT3 TKI exposure and in 28 (80%, CR in 11%) of 35 patients with prior TKI exposure. The median OS was 10.6 months and 9.6 months, respectively. Grade 3 or 4 AEs occurred in 97% of patients, mostly characterized by cytopenias (80%). AEs led to venetoclax and gilteritinib interruptions in 51% and 48% of patients, and to discontinuation of treatment in 15% and 13%, respectively. Serious AEs occurred in 75% of patients, most commonly febrile neutropenia (44%) and pneumonia (13%) [31]. This combination approach produced a highly modified composite CR rate in patients with FLT3-mutated R/R AML; however, dose interruptions for cytopenias were very common and this regimen showed a high toxicity profile.

The addiction of gilteritinib to azacitidine and venetoclax in FLT3-mutated AML was another fascinating triplet combination. In the phase I/II trial recently reported by Short et al., the ORR was 100% (27/27) with a 92% CR in newly diagnosed patients, a median OS that had not yet been attained, and an OS of 85% at 1-year. In R/R patients, the ORR was 70% (14/20), with a CR rate of 20% (4/20), and a median OS of 5.8 months. With a median OS of 10.5 months, outcomes were better in patients who had not previously received gilteritinib or venetoclax [32].

6.3. Gilteritinib plus chemotherapy in patients with newly diagnosed AML

Recently, encouraging data on the association between gilteritinib and induction and consolidation chemotherapy were presented at the 10th Annual Meeting of the Society of Hematologic Oncology. Patients enrolled in this phase 1 trial (NCT02236013), were required to be at least 18 years of age with newly diagnosed AML and have an ECOG performance status of 2 or less; the presence of a *FLT3* mutation at baseline was not required. Dose escalation of gilteritinib was assessed in the part 1 of the study to identify the MTD. Induction regimen provided 3 days of idarubicin with 7 days of cytarabine and 14 days of gilteritinib at doses of 20 mg, 40 mg, 80 mg, 120 mg, or 200 mg, given on days 4 through 17 for up to 2 cycles. Consolidation approach included high-dose cytarabine plus the same dose of gilteritinib given daily for the first 14 days of each cycle for up to 3 cycles. Finally, patients received maintenance treatment based on gilteritinib daily for 28 days for up to 26 cycles. The dose expansion study (part 2) provided gilteritinib at 120 mg a day, with induction, consolidation, and maintenance following the same treatment pattern as dose expansion trial. In part 3 of the study, the gilteritinib dosing-schedule during induction was modified to begin with the completion of chemotherapy, running from days 8 through 21, and the other receiving 3 days of daunorubicin and 7 days of cytarabine. Consolidation and maintenance followed the same treatment pattern as parts 1 and 2. In part 4 of the study, gilteritinib was given up to 56 consecutive days during consolidation. A total of 79 patients were enrolled; among them, 56.4% of patients harbored *FLT3* mutations, 42.3% had *FLT3*-ITD mutations, and 41% had *FLT3*wt disease. At the end of treatment, the composite CR in patients with *FLT3* mutation was 90.9% with 70.6% of patients who

achieved a CR. The 26-week, 1-year, and 2-year OS rates were 92.4%, 82.1%, and 69.2%, respectively, in this subgroup. Additional data showed that while censoring for HSCT, the median disease-free survival (DFS) for patients with FLT3 mutations (n = 40) was 460 days (95% CI, 150-970), while FLT3-negative population (n = 22) experienced a median DFS of 288 days (95% CI, 23-971). The MTD of gilteritinib was established to be at 120 mg per day, and dose-limiting toxicities occurred in 15 of 78 (19.2%) patients given gilteritinib. AEs led to the discontinuation of gilteritinib in 24.4% of patients. Grade ≥ 3 treatment-emergent AEs were reported in 93.6% of patients [33]. According to these results, an effective anti-leukemic response was observed in terms of CR and OS, particularly in the FLT3 mutated subgroup in newly diagnosed AML who received gilteritinib in combination with intensive chemotherapy. These data support further trials to confirm the validity of this approach and to compare this regimen with the already approved treatment based on the combination of midostaurin with intensive chemotherapy in FLT3 mutated patients. **Table 3** summarizes the ongoing and recruiting studies including gilteritinib in combination with chemotherapy or other small molecules in R/R and *de novo* AML.

Table 3. Ongoing and recruiting studies including gilteritinib.

Number of the study	Protocol regimen	Eligible patients
NCT04027309	gilteritinib versus midostaurin in combination with induction and consolidation therapy followed by one-year maintenance	newly diagnosed acute myeloid leukemia or myelodysplastic syndromes with excess blasts-2 with FLT3 mutations
NCT04140487	azacitidine, venetoclax, and gilteritinib	relapsed/refractory FLT3-mutated acute myeloid leukemia, chronic myelomonocytic leukemia, or high-risk myelodysplastic syndrome/myeloproliferative neoplasm
NCT04240002	gilteritinib combined with chemotherapy	children, adolescents and young adults FLT3-ITD positive relapsed/refractory acute myeloid leukemia
NCT05546580	iadademstat and gilteritinib	relapsed/refractory acute myeloid leukemia with FLT3-ITD mutation
NCT05520567	gilteritinib, venetoclax and azacitidine	newly diagnosed with acute myeloid leukemia with FLT3 mutations
NCT05028751	lanraplenib (lanra) in combination with gilteritinib	FLT3 mutated relapsed/refractory acute myeloid leukemia
NCT05010122	astx727, venetoclax, and gilteritinib	newly Diagnosed, relapsed/refractory FLT3-Mutated acute myeloid leukemia or high-risk myelodysplastic syndrome
NCT04293562	standard chemotherapy versus therapy with cpx-351 and/or gilteritinib	newly diagnosed acute myeloid leukemia with or without FLT3 Mutations
NCT05010772	decitabine alone or in combination with venetoclax, gilteritinib, enasidenib, or ivosidenib as maintenance therapy	acute myeloid leukemia in remission

7. Maintenance therapy with gilteritinib after allogenic transplant

To date, there are currently no definitive results deriving from randomized trials to validate the use of gilteritinib for post HSCT maintenance therapy. The pivotal Astellas-sponsored MORPHO trial, addressing the value of a gilteritinib maintenance therapy post HSCT is currently ongoing with results being expected in 2025 (NCT02997202) [34]. However, recently ASTELLAS announced that since relapse free survival (RFS) was not statistically significant at the primary analysis, the study, including follow-up, will be stopped as per the study protocol. (news provided by Astellas Pharma Inc.; BMT CTN on March 2023). The BMT CTN 1506 is a randomized, phase III trial aimed to assess maintenance with gilteritinib versus placebo after HSCT in patients with FLT3-ITD mutated AML who achieved first CR (NCT02997202). Gilteritinib is given between days 30 to 90 after HSCT at 120 mg daily for 2 years. The study provides a deep-sequencing assay strongly sensitive for FLT3-ITD mutations for minimal residual disease testing which will identify patients most likely to respond to maintenance approach with gilteritinib [34,35].

In the ADMIRAL study, 20% (49 of 247) of patients enrolled in the gilteritinib arm and 10% (14 of 124) of patients treated with salvage chemotherapy were alive for ≥ 2 years. Among the patients still alive, 18 of 49 underwent HSCT and 16 continued gilteritinib as post-transplant maintenance treatment. Post-HSCT maintenance with gilteritinib resulted in improved OS and RFS, similar to the findings of prior studies with other FLT3 inhibitors [18]. Among patients in the study receiving gilteritinib for maintenance, OS at 24 months was 96.2% compared with prior reports of 90.5% with sorafenib and 85% with midostaurin [36–38]. The RFS in the gilteritinib maintenance group was 89.7% at 24 months compared with prior reports of 85% with sorafenib and midostaurin [36–38]. Furthermore, several factors correlated with worsened graft-versus-host DFS and RFS, including matched unrelated donor transplant, pre-transplant anti-thymocyte globulin, and lack of maintenance FLT3 inhibitors [18]. Recently, Perl et al. [39] reported data on patients included in the ADMIRAL study who underwent HSCT and received gilteritinib after transplantation as maintenance therapy. Patients in the gilteritinib arm proceeding to HSCT could receive post-transplantation maintenance with gilteritinib if they were within 30 to 90 days' post-transplantation and had achieved CRc with effective engraftment and no post-transplantation complications. The OS rates at 12 and 24 months were 68% and 47%, respectively, for all transplant recipients. Even though there was a tendency for prolonged OS following pre-transplant CRc, post-transplant survival was equivalent in the 2 arms. Following HSCT, patients who restarted gilteritinib showed low rates of pre-transplantation CRc (20%) or CR (0%) recurrence. Increased ALT level (45%), pyrexia (43%), and diarrhea (40%), as well as grade 3 AEs, were the most frequently reported AEs with post-transplant gilteritinib. Grade 3 acute graft-versus-host disease occurrences and associated mortality were infrequent. Overall, post-transplantation survival in the 2 study arms was comparable [39]. Recently the MD Anderson group reported data of a retrospective analysis of adult patients with FLT3-ITD AML who underwent HSCT and thereafter received sorafenib or gilteritinib as post-transplant maintenance. A total of 55 patients were treated with either gilteritinib (n=27) or sorafenib (n=29); median time to initiation of gilteritinib was 60 days after transplant and median duration of time on gilteritinib was 385 days. The 1-year progression-free survival (PFS) (66% versus 76%; p =0.4) and relapse incidence (19% versus 24%; p =0.6) were similar between the two groups, respectively; the 1-year OS (78% versus 83%; p =0.4) was also comparable. However, non-relapse mortality at 1 year was higher in the gilteritinib group (15% versus 0%; p= .03) [40]. Also, the Japanese group retrospectively analyzed 25 FLT3-mutated R/R AML patients who received HSCT (14 patients received gilteritinib as maintenance therapy and 11 patients did not). The median time from transplant to the initiation of gilteritinib was 36 days, while the median starting dose was 40 mg (range 20-120 mg). Patients treated with gilteritinib showed significantly longer 1-year leukemia free survival (100% versus 36.4%; p = .0028) and 1-year OS (100% versus 45.5%; p = .0075) than those without gilteritinib. Among patients showing positive minimal residual disease (MRD) or a non-complete response before transplant (n=19), those on gilteritinib maintenance showed a lower 1-year cumulative incidence of AML relapse (0% versus 68.8%; p = .0028) [41]. These results support the hypothesis that gilteritinib

maintenance therapy might prevent disease relapse after transplant especially in those patients with positive MRD at the time of HSCT.

8. Gilteritinib for extramedullary AML relapse

The FLT3-ITD gene mutation has been described to promote leukemic cell infiltration into visceral organs while inhibiting homing to the bone marrow by downregulation of CXCR4 signaling [42]. Several studies have demonstrated that miRNAs may promote hematopoiesis and hematological diseases [42, 43]. FLT3 mediated signaling controls the expression of several miRNAs, with both downregulation (miR-451 and miR-144) and upregulation (miR-155, miR-10a, and miR-10b) mechanisms. The expression of these small molecules seems to favor extramedullary blasts infiltration although underlying mechanisms remain yet to be demonstrated [43]. Several case reports have described the efficacy of gilteritinib in patients with FLT3 mutant extramedullary relapse before or after transplant. Perrone et al. first demonstrated the potential biological effect of gilteritinib within the central nervous system (CNS) [44]. They described the case of a therapy related AML with FLT3-ITD and NPM1 mutation who presented an extramedullary relapse involving the CNS after having achieved a complete response with CPX-351. The disease rapidly progressed despite a brief second response to treatment with medicated lumbar puncture and FLA-Ida chemotherapy. For this reason, the patient received gilteritinib and after 3 months of treatment, even if only a bone marrow partial response was attained, the meningeal localization completely disappeared [44]. Moreover, Vignal et al., reported the presence of gilteritinib in cerebral-spinal fluid at therapeutic doses [45]. In another case [46], a 38-year-old male patient with myelodysplasia-related changes and FLT3-ITD mutated AML underwent HSCT after achieving CR with standard chemotherapy. On day + 400, the patient experienced a right supraclavicular mass with simultaneously occurring AML blasts in the bone marrow. Both extramedullary and medullary blasts presented FLT3-ITD mutation. Therapy with 120 mg/day of gilteritinib was started determining a medullary and extramedullary CR and allowing the patient to proceed to a second HSCT [46]. Furthermore, gilteritinib seems to have efficacy also in infrequent localization of AML. Kim et al. [47] described a case of a FLT3-ITD mutated patient who presented an AML relapse involving the temporal iris, ciliary body, and choroid by a leukemic infiltrative mass. The patient started treatment with oral gilteritinib obtaining rapid regression of the tumor, complete disappearance of the iris involvement, and an important reduction of the ciliochoroidal mass associated with a significant improvement of the visual acuity [47]. The mechanisms underlying the documented activity of gilteritinib in extramedullary AML are still unknown, however this small molecule appeared to hold efficacy in this setting and should be taken into consideration especially in heavily pretreated patients.

9. Antifungal prophylaxis in patients treated with gilteritinib

Due to the fact that gilteritinib mainly undergoes CYP3A4-dependent metabolism, the manufacturer advises against using gilteritinib concurrently with drugs that strongly induce or inhibit CYP3A4 and instead suggests to select alternative treatments [15]. In the phase I/II CHRYSLIS trial, which examined possible drug-drug interactions between gilteritinib and moderate and strong CYP3A4 inhibitors (such as fluconazole, voriconazole, and posaconazole), gilteritinib exposure was found to be less than two times higher when an azole was also administered. The incidence of AEs did not vary between patients who received a moderate or strong CYP3A4 inhibitor and those who did not, hence this increase was not deemed to be clinically relevant [17]. The effects of weak CYP3A4 inhibitors (such as itraconazole) and strong CYP3A4 inhibitors (such as fluconazole) on the pharmacokinetics of gilteritinib were assessed in an open-label drug-drug interaction research. The findings showed that fluconazole was associated with a smaller increase in systemic exposure of gilteritinib (1.43-fold) compared to itraconazole (2.3-fold), which was linked with a significant increase in systemic exposure of gilteritinib [15]. The larger phase III ADMIRAL trial, however, forbade the use of posaconazole, itraconazole, and voriconazole, leaving unaddressed the issue on how to combine these drugs [8]. Aleissa et al. assessed the prevalence of AEs associated with gilteritinib in 47 patients who received gilteritinib either with or without antifungal triazoles. In

the gilteritinib-triazole group, AEs related to gilteritinib were comparable to those in the gilteritinib group without triazole (75% versus 55.5%, $p = 0.23$). The severity of AEs, dose reductions or discontinuations from gilteritinib (15% versus 14.8%), and 90-day mortality (35% versus 11.1%) were also comparable between the two groups [48]. However, how interactions between azoles and gilteritinib impact on toxicities is not yet fully defined. Therefore, the European Hematology Association guideline on antifungal prophylaxis in patients with AML treated with novel-targeted therapies recommended triazole antifungal prophylaxis in patients treated with gilteritinib only in those heavily pretreated [49].

10. Development of resistances to gilteritinib

Around 30% of patients who relapse after achieving a remission to type 1 FLT3 inhibitors (midostaurin, gilteritinib and crenolanib) carry mutations in the *RAS* pathway, making it the most prevalent mutation-derived mechanism of resistance to type 1 inhibitors. These mutations may appear as new mutations following therapy or as clonal proliferation with rising variant allele frequency (VAF) over the course of therapy [50]. Poorer outcomes in both primary and secondary relapse scenarios are linked to higher VAFs in *RAS/MAPK* mutations. *RAS* pathway mutations are less common with type 2 FLT3 inhibitors (quizartinib) than with type 1 inhibition, occurring in just 6% of patients relapsing after type 2 inhibitors. *RAS*-mutated clones can spread in patients using quizartinib, even though FLT3-TKD mutations are the most common route of resistance to type 2 inhibitors [51]. It was hypothesized that preservation of FLT3 mutant clones can also depend on the bone marrow microenvironment (BMME). Indeed, soluble cytokines and growth factors together with cell-cell contact between leukemic cells and stromal cells within BMME can act as a mediator for the preservation of leukemic clones [52]. BMME adaptation and changes have been described along therapy. Patients relapsing after intensive chemotherapy courses were found to have considerably greater FLT3 ligand levels, inducing AKT, ERK, and other pro-apoptotic proteins downregulation through FLT3 ligand-FLT3wt binding. Despite FLT3-ITD inhibition, FLT3wt mediated activation of these pathways promotes leukemic cell survival [53].

In the ADMIRAL study, 40 patients acquired new mutations during treatment. Among them, in 18 patients the *RAS/MAPK* pathway was affected, while FLT3 was involved in 6 cases (5 patients presented the F691L mutation); 3 had *WT1* (one had the F691L mutation), 1 had *IDH1*, and 1 had *GATA2*. Thirteen patients (32.5%) had no new mutations. During relapse, FLT3 *F691L* gatekeeper mutations and mutations in the *RAS/MAPK* pathway genes were mutually exclusive [54]. *RAS/MAPK* and FLT3 *F691L* mutations were acquired by non-transplanted patients during relapse, however the latter did not correlate with refractoriness. Uncertainty exists regarding the relationship between the dosage of gilteritinib and the prevalence of emergent FLT3 *F691L* gatekeeper mutations at relapse. In the ADMIRAL study, patients who received gilteritinib at 120 mg/day had a comparable incidence of FLT3 *F691L* as seen in relapsed patients who received gilteritinib from 20 to 200 mg/day, but none of the patients receiving >200 mg/day acquired this kind of mutation at relapse. However, compared to other patients, those receiving 120 mg/day had improved OS [55]. Another study demonstrated a relationship between gilteritinib dose and occurrence of resistance in 22 FLT3 mutated patients analyzed at relapse by next generation sequencing and single cell analysis, reporting a more likely onset of *RAS* or FLT3 *F691L* mutations in those treated with doses below 200 mg [56].

Recently, it was reported that FF-10101, a selective and irreversible FLT3 inhibitor, significantly inhibited FLT3-ITD and -TKD mutations, including *F691L* and *D835*, both *in vitro* and *in vivo* [57,58]. Fifty-two patients with R/R AML were enrolled in a phase I dose escalation study to test the inhibitor. In pretreated patients (median number of prior therapies, $n=3$), continuous treatment with FF-10101 at a dose of 10-225 mg four times per day or 50-100 mg twice daily led to a composite CR rate of 13% and a partial response rate of 8%, including those with activating FLT3-TKD mutations resistant to gilteritinib and other FLT3 TKIs. Well-tolerated doses of 50-75 mg twice daily resulted in long-lasting FLT3 suppression. The trial is still ongoing, but not recruiting patients [59].

Sitravatinib is a multi-kinase inhibitor under evaluation in ongoing clinical trials of several solid tumors. In a recent study, it was explored the antitumor activity of sitravatinib against FLT3-ITD and

clinically-relevant drug resistance in FLT3 mutant AML. The FLT3-ITD-*F691L* mutation caused resistance to gilteritinib and all other FLT3 inhibitors, both *in vitro* and *in vivo*, whereas sitravatinib showed a potent inhibitory impact. With stronger and more consistent suppression of p-ERK and p-AKT than gilteritinib, sitravatinib maintained excellent efficacy against FLT3 mutation in the presence of cytokines. Additionally, sitravatinib was more effective against patient blasts carrying FLT3-ITD *in vitro* and in the PDX model than gilteritinib [60].

11. Conclusions

Gilteritinib is an easy-to-use oral drug, with toxicities mainly represented by hematologic myelosuppression and high liver enzymes. Particular mention should be given to the promotion of differentiation of leukemic blasts in a sizeable subset of R/R FLT3 patients [61], that has also been reported in patients treated with IDH-mutant AML treated with the IDH inhibitors (enasidenib and ivosidenib), for the induction of QT prolongation, pancreatitis, embryo-fetal toxicity, and a rare neurologic complication: posterior reversible encephalopathy syndrome (PRES), which requires permanent discontinuation of the drug.

From the clinical point of view, gilteritinib has improved response and survival rates in comparison to different standard salvage chemotherapy regimens in the R/R AML setting. In the ADMIRAL trial, the median OS was almost double for gilteritinib (9 months versus 5 months for standard chemotherapy). Indeed, gilteritinib represents a clinical upgrade since patients with a primary refractory disease (i.e., refractory to standard induction and high dose cytarabine), who carry a FLT3 mutation are shifted to an oral drug that has fewer side effects and is more effective than conventional chemotherapy. This approach is so appealing that almost all patients with a relapsed AML are currently re-tested for FLT3 mutations [62], even if patients acquiring an FLT3 mutation at relapse represent a minority and its occurrence has been reported in less than 8% [63]. Moreover, the biology of FLT3 mutation is complex: although always leading to an in-frame transcript, FLT3-ITD can vary in sequence and length (between 3 and > 400 nucleotides), and despite the prognostic relevance of the allelic ratio, which corresponds to the size of the mutated clones carrying FLT3-ITD [64], there is no standardized cut-off value in the allelic ratio when prescribing (or not prescribing) gilteritinib to small clones. Indeed, patients with a high FLT3-ITD allelic ratio (≥ 0.77) showed a longer OS (7.1 versus 4.3 months), and other co-mutations in FLT3 molecular partner retain a prognostic impact [19]. However, searching for several co-mutations at relapse is currently unpractical, and methodological issues remain to be addressed regarding the standardization of the FLT3-ITD allelic ratio assay [62]. We summarized the results of different real-life studies of gilteritinib that confirmed that patients treated in daily clinical practice attain results similar to patients randomized in the ADMIRAL trial [21–24].

At 2 years from the start of gilteritinib, only 26 (20%) patients survived in the ADMIRAL trial and most of them (18) underwent HSCT as consolidation [18]. These data suggest that gilteritinib represents an excellent bridging therapy to allotransplant, and patients who continue gilteritinib often develop resistance by several mechanisms. As for the setting of maintenance after HSCT, the role of gilteritinib remains uncertain after the termination of NCT02997202 trial [34]. More data are eagerly awaited to shed definitive light on this topic. Indeed, an intriguing question is raised by the clinical efficacy showed by sorafenib [65], which, albeit not active against FLT3-TKD, clearly outperforms gilteritinib as maintenance in post-HSCT setting. Unfortunately, the possibility of starting gilteritinib as a pre-emptive strategy only in patients who manifest a minimal residual disease positivity after HSCT (similarly to acute lymphoblastic leukemia Philadelphia positive [66]), is hampered by technical difficulties to exactly quantify FLT3 mutation [67]. Indeed, the consensus document from the European Leukemia Net minimal residual disease Working Party states that mutations in signaling pathway genes (FLT3-ITD, FLT3-TKD) most likely represent residual AML when detected, but are often sub-clonal and have a low negative predictive value; these mutations are best used in combination with additional minimal residual disease markers [68].

The future developments of gilteritinib in the treatment of FLT3-mutated AML patients will also depend on the pending results of its association with other drugs. As reviewed, the combination with

intensive chemotherapy for *de novo* AML is under study, but this field is already covered by midostaurin [36] and quizartinib [69], thus strongly limiting expectations for real innovation. The association of gilteritinib with hypomethylating agents (mainly with azacitidine) has been disappointing [25]. Finally, the combination with venetoclax produced modest improvement, but at the cost of elevated hematological toxicity [31].

In the meanwhile, we have gathered increased experience to deal with challenging presentations of AML, like extramedullary localization of myeloid sarcoma, where gilteritinib seems to have a role, and in patients presenting an invasive fungal infection. Nowadays, the most challenging issue in patients treated with gilteritinib remains how to overcome the occurrence of resistance. Resistance to TKI is common in several cancers and represents an evolutionary response to a selective pressure exerted at a sub-clonal disease level. In the gilteritinib arm, the median duration of CR was 23.0 months; the median durations of CRc and CR/CRh were 4.6 months and 10.0 months, respectively [18]; these data indicate that patients who achieve better hematological responses, experience prolonged clinical benefit, while for resistant patients outcome is extremely poor.

We discussed the current state-of-art of gilteritinib studies and evaluated the advantages and limitations of its use in R/R AML. Overall, these data are highly encouraging and open a new avenue to the further development of targeted therapy approaches in FLT3-mutated AML.

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