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Gilteritinib Clinical Activity in Relapsed/Refractory *FLT3* Mutated AML Previously Treated with *FLT3* inhibitors.

Running Title: Gilteritinib use in previously treated *FLT3* + AML

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Abstract

Gilteritinib is approved for the treatment of relapsed/refractory (R/R) acute myeloid leukemia (AML) with a FLT3-mutation ($FLT3^{mut+}$). However, the gilteritinib phase 3 ADMIRAL study (Perl et al NEJM 2019) was conducted prior to widespread adoption of either midostaurin as a component of standard intensive induction and consolidation or post-transplant FLT3 inhibitor maintenance. We performed a retrospective analysis using data from 11 US centers and where we identified 113 patients who received gilteritinib alone or as combination therapy for the treatment of R/R $FLT3^{mut+}$ AML. The composite CR rate (CRc, defined as CR + CRi + CRp) was 48.7% (n= 55). The CRc rate after treatment with gilteritinib in patients who were treated with only prior 7+3 and midostaurin with or without consolidation was 58% with a median survival of 7.8 months. Survival was longest in patients who obtained a CR, particularly a cMRD (Clinical minimal or measurable residual disease) negative response; this remained significant after censoring at the time of SCT. MAPK pathway activating mutations that are known for gilteritinib resistance (NRAS, KRAS, and PTPN11) had lower CRc (35% vs 60.5%) and lower mOS than patients' whose leukemia did not express these mutations (4.9 months vs 7.8 months) (HR 2.4- 95% CI 1.1-5.4) p value <0.01.

Keywords: AML, FLT3 mutation, FLT3 inhibitor, gilteritinib

Introduction

Mutations in *FLT3* occur in approximately 30% of patients with new acute myeloid leukemia (AML) and activate intracellular tyrosine kinase signaling to promote cellular proliferation, impair differentiation, and inhibit apoptosis¹⁻⁴.

Historically, FLT3 mutations were associated with early relapse and poor survival with traditional salvage therapy options. Several FLT3 tyrosine kinase inhibitors (FLT3i) have been developed for the treatment of FLT3^{mut} AML^{5,6}. The RATIFY trial demonstrated that the addition of midostaurin to induction chemotherapy improved the overall survival and led to the approval of midostaurin in combination with daunorubicin and cytarabine for new diagnosis of FLT3^{mut} AML⁷. Randomized studies afterwards showed a substantial reduction in post-transplant relapse and improved survival from the use of midostaurin and sorafenib as post-transplant maintenance in FLT3-ITD mutated patients⁸⁻¹⁰.

The ADMIRAL trial was a phase 3 randomized controlled trial that compared the second generation FLT3i gilteritinib with salvage chemotherapy for the treatment of relapsed or refractory (R/R) FLT3^{mut} AML. 371 patients were randomized 2:1 to either single agent gilteritinib or their treating physician's pre-randomization choice from 4 standard salvage chemotherapy regimens. The study met its primary endpoint by

showing that survival was superior in the gilteritinib arm, with a median overall survival of 9.3 months, compared to from 5.6 months with chemotherapy (HR 0.64, $p < 0.001$). However, the ADMIRAL trial enrolled patients prior to widespread use of frontline FLT3is and indeed, only 12.4% of the study population had previously been treated with a FLT3i¹¹. Thus, the benefits of gilteritinib in the context of modern therapy incorporating frontline FLT3i's is not well characterized. Additionally, therapy with FLT3i has been shown to drive expansion of clones with additional on-target mutations, such as FLT3-TKD mutations that may confer resistance to subsequent FLT3i¹² including prior sorafenib therapy¹³. Similarly, other FLT3 mutations, including N676K and F691L, may be associated with resistance to midostaurin or gilteritinib, respectively¹⁴⁻¹⁵. A more common pattern of resistance to gilteritinib is not on-target mutations in FLT3, but off-target emergent mutations in RAS/MAPK pathway genes, including *N-RAS* or *K-RAS*, as well as *PTPN11*. Treatment emergent mutations in these genes have also been described at progression after frontline intensive chemotherapy with midostaurin¹⁶. For these reasons, we sought not only to describe the clinical responses but to clarify whether pretreatment mutations contributed to the response or survival with gilteritinib salvage in patients who have received prior FLT3is and whether such mutations might help inform therapy choice as to single agent vs. combination treatment for R/R FLT3mut+ AML.

Methods:

We performed the largest multi-institutional retrospective analysis from 01/2020 – 06/2021 from 11 US cancer centers of patients who had received a prior FLT3i and then received gilteritinib alone or as combination therapy for the treatment of R/R FLT3^{mut+} AML. Patients were excluded from the analysis if they received gilteritinib: (1) as a part of an ongoing and not reported trial (2) as a maintenance post-transplant with no documented relapsed disease prior to start of gilteritinib (3) because of intolerance to a prior FLT3i due to side effects in a patient in marrow remission (4) as frontline therapy with no prior FLT3i exposure. Patient demographic data as well as disease characteristics were collected including: date of diagnosis, date of relapse date of last follow up or death, type of treatment at diagnosis and relapse, complete blood counts at diagnosis, mutational landscape at diagnosis and relapse, and clinical minimal residual disease evaluation. AML risk stratification was assessed based on 2017 European leukemia net (ELN) classification¹⁷.

Response criteria were identical to the ADMIRAL trial, which used a modified version from the international working group response definitions¹⁸. Complete remission (CR) was defined as a morphologic leukemia-free state, a bone marrow regenerating normal hematopoietic cells with a normal marrow differential and < 5% blasts, and peripheral blood counts showing an absolute neutrophil count (ANC) $\geq 1 \times 10^9$ /L, platelet count $\geq 100 \times 10^9$ /L, transfusion independence for at least one week, and no circulating or extramedullary blasts. Complete remission with incomplete platelet recovery (CRp) was defined as CR except for

incomplete platelet recovery ($< 100 \times 10^9 /L$). Complete remission with incomplete hematological recovery (CRi) was defined as CR except for residual neutropenia $< 1 \times 10^9 /L$ with or without complete platelet recovery. Transfusion independence was not required for CRi. Composite Complete Remission (CRc) was defined as the combined rate of all CR, CRp and CRi. The investigators from each institution assessed the gilteritinib response on their own patient cohort independently based on the criteria mentioned above. This was done as patient data from each institution was de-identified before it was transferred and combined with the rest of the data-set.

Clinical minimal or measurable residual disease (cMRD) was evaluated on bone marrow flow cytometry (MFC) using a cutoff of $<1 \times 10^3$ cells or polymerase chain reaction (PCR) for FLT3 mutation with a minimum sensitivity of 5%. This cMRD assessment was included as the depth of response and was able to be determined at all the centers.

Survival from the time of gilteritinib initiation till death or loss to follow-up was recorded in months. Descriptive statistics were conducted to assess patients' characteristics; t test was performed on continuous variables, and multivariate analysis included all variables collected to determine interaction with patient outcome (response rates and survival in months) and analyses were adjusted for multiple comparisons. Kaplan-Meier curves and log rank test were used for survival analysis after gilteritinib initiation. A p-value of <0.05 was considered statically

significant. Institutional review board approval and data transfer agreements were obtained from the participating cancer centers.

Results

410 patients were evaluated from 11 cancer centers and 113 patients were eligible based on the selection criteria. Patient characteristics are shown in Table 1. The median age of the sample was 58.3 years with a range of 18-92 years. 66 patients were white (58.4%) and 65 females (57.5%). 73 patients (64.6%) had disease that was characterized as favorable or intermediate risk AML by ELN (4 patients had missing NGS or cytogenetic information). ITD was the most common FLT3 mutation subtype (84.1 %) and 9 patients (8%) had both mutations (ITD and TKD). The majority of the patients had received midostaurin (57.5%) as a prior FLT3i followed by sorafenib (30%). In this cohort, 49 patients (43.4%) had a history of allogeneic stem cell transplant (ASCT) prior to receiving gilteritinib and 24 patients (21.2%) underwent ASCT post gilteritinib therapy. The mean duration of gilteritinib therapy was 4.6 months with a range of 0.2 to 25 months. The majority of patients received gilteritinib as a single agent therapy (62.8%) while the remaining patients received gilteritinib in combination with other agents as shown in Table 2. The mutational landscape at diagnosis and relapse done via next generation sequencing (NGS) were available in 104 patients (92%) and shown in Figure-S1(A&B).

55 of 113 (48.7%) patients in our cohort achieved a CRc with CR in 25 patients (22.1%) and CRi+ CRp in 30 patients (26.5%). The median overall survival (mOS) for all patients was 7.0 months (SD \pm 0.7 months). The CRc rate based on the prior FLT3i was comparable: 53.9% CRc rate in patients who received midostaurin vs 41.2% in patients who received sorafenib (Figure-S2). This was not statistically significant. There was no difference in mOS between prior midostaurin and sorafenib (7.8 months vs 5 months $p = 0.2$). In a subset analysis of patients treated with 7+3 + midostaurin induction with or without consolidation (RATIFY regimen), gilteritinib resulted in a CRc rate of 58% and a mOS of 7.8 months. A trend toward higher CRc rate was noted in patients treated with gilteritinib in combination regimens ($n=26$) rather than as a single agent ($n=30$) (64% vs 43%, respectively, $p=0.09$ using Chi-square test), however, no survival advantage for combination therapy was seen over single agent. While mOS was not different in patients who underwent hematopoietic stem cell transplant (HSCT) before initiation of gilteritinib therapy (7.4 months for transplant group vs 7.1 months for none-transplant), patient who underwent HSCT after gilteritinib had a statistically significant improvement in mOS compared to patients who did not receive transplant; 12 months vs 5.2 months HR = 0.46 (95% CI 0.2-0.6) Figure-1a.

Once post-gilteritinib HSCT data was censored, survival analysis was performed based on disease response. Patients who achieve CR had the best overall survival compared to CRi + CRp and non-responders (NR).

After a median follow up of 9 months, the mOS in CR group was not reached compared to mOS of 7.4 months in CRi+CRh and 4.3 months in NR, this was statistically significant with a p value of 0.01 using log rank test (the median follow up for the whole cohort is 11 months). In CR group, patients who achieved MRD-negative status by either MFC (n=11) or by PCR (n=16) had improved median OS compared with their respective MRD positive counterparts (Figure-1b). Patients with cMRD negative by both MFC and PCR (n=11) had 100% probability of survival. Dual modality MRD negativity was statistically significant in comparison to negative cMRD by only one method (MFC or PCR) (p =0.0001).

We evaluated the mutational status at diagnosis or relapse in its relation to response and survival analysis. Overall survival was independent of ELN risk group with favorable/intermediate ELN vs poor risk (6.7 months vs 4.3 months p = 0.3). Further analysis showed that FLT3 mutation type (ITD vs TKD) had no effect on gilteritinib response or overall survival (6.8 months vs 7.1 months). The two most common co-mutations (*NPM1* and *DNMT3A*) did not affect response or survival in this analysis including a double positive mutation status. We noted the persistence of ASXL1 (N=9 at diagnosis and N= 6 at relapse), TP53 (N=3 at diagnosis and 2 at relapse) and RUNX1 (N=15 at diagnosis and N= 12 at relapse) mutations at diagnosis and relapse and these mutations did not affect mOS compared to wild type for each mutation (5.7 months vs 7.1 months) Figure-1c. Interestingly, we also noted a new emergence of WT-1 mutation at relapse (N=11), however, emergence of this mutation did not

affect response or overall survival. On the other hand, mutations that activate the MAPK kinase pathway and have been involved in gilteritinib resistance such as NRAS and PTPN11 were noted pre-gilteritinib therapy in (N=19). Presence of these mutations had a nonsignificant inferior impact on CRc rate (59% vs 37.5%) and mOS (4.9 months vs 7.8 months) (HR 2.4- 95% CI 1.1-5.4 -p =0.0057) (Figure-1d).

Discussion

This is the first and largest real-world study to confirm that gilteritinib retains its clinical activity in the setting of a prior FLT3i treatment. This multi-institutional analysis provides critical clinical information about the sequencing of different FLT3i outside of a clinical trial setting. In this data set, patients remained on gilteritinib for an average of 5.7 months and their chance of achieving CRc rate was 48.7% which is slightly lower from what is reported in ADIMIRAL trial (54%) but this study provides a real-world experience with FLT3i sequencing. Furthermore, an important clinical question that we addressed is CRc rate in patients with FLT3^{mut+} AML who relapse on 7+3 + Midostaurin, with or without consolidation treatment. In this subgroup, gilteritinib produced a CRc rate of 58% and a mOS of 7.8 months. Overall, this is encouraging as our study exclusively included patients that relapsed or progressed on a prior FLT3i and drug resistance is always of a considerable fear for both the patients and physicians. Furthermore, multivariate analysis showed that FLT3 mutation type, ELN risk and type of prior FLT3i used before did not affect the CRc rate and mOS. The patients who achieve CR had the best

mOS out of CRc and most notably, cMRD negative by both PCR and flow cytometry had 100% probability of survival with a median follow up of 11 months. This is an interesting finding that requires validation in additional studies as a duration of gilteritinib therapy is not known and whether patients can eventually safely discontinue gilteritinib without undue risk of relapse is unclear. Typical of other studies in R/R AML, we also show that use of HSCT is associated with prolonged mOS.

Our study is unique as it provides insights on the mutational landscape for these patients at diagnosis and relapse. Previous studies show that *NPM1* and *DNMT3A* mutations status affect prognosis in patients with *FLT3*^{mut+} AML. We show that *NPM1* and *DNMT3A* mutations status – while very prevalent in our patient population- did not affect gilteritinib response or mOS reflecting that relapsed or refractory AML has a poor prognosis regardless of these mutations. A trend toward worse outcome was noted in *ASXL1*, *TP53*, and *RUNX1* mutations, however, their prevalence was relatively low. Despite the emergence of WT-1 at relapse, this mutation at baseline had no effect on response or survival. We also interrogated various mutations that have been associated with gilteritinib resistance in other reports, such as mutations in the MAPK pathway. In this analysis, we confirm that *NRAS* and *PTPN11* mutations – while seldom mutated at baseline- appear to be associated with worse survival compared to patients lacking these mutations. Thus, they may confer resistance to gilteritinib and thus alternative regimens for the

treatment of patients and may warrant consideration of combination strategies or alternate regimens through clinical trials.

This retrospective analysis has some limitations. Most notably, the cMRD testing which was defined clinically, was done at different institutions and not verified in a central lab. We acknowledge that it is not the standard sensitive MRD testing and the depth of MRD assessment is limited with sensitivities of these assays. However, these testing techniques have been ordered and interpreted by the treating physicians outside of clinical trial setting and incorporating them in our analysis did provide valuable clinical information. In addition, although our data included patients from 11 cancer centers, only five patients were African Americans which supports health and ethnic disparities that tertiary cancer centers face. This is very important issue that needs to be addressed in future clinical trials.

In conclusion: this multi-center analysis is the first to show that gilteritinib remains a clinically active agent after treatment failure of prior FLT3i's, including in patients treated with the approved 7+3+ midostaurin regimen. We also confirm that mutations affecting the MAPK kinase pathway such as NRAS and PTPN11 appear contribute to resistance to gilteritinib. Finally, factors associated with long-term survival to gilteritinib salvage include use of subsequent transplant, FLT3 mutation clearance, and achievement of an MRD negative state by flow cytometry.

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Figure Legends:

Figure-1a: Survival analysis via KM curves based on stem cell transplant status. Survival is measured in months.

Figure-1b: Survival analysis via K-M curves based on clinical minimal residual disease (cMRD) testing. Flow refers to flow cytometry and PCR refers to FLT3 testing via polymerase chain reaction.

Figure-1c: Survival analysis via K-M curves based on any of three following mutations: ASXL, TP53 and RUNX1

Figure-1d: Survival analysis via K-M curves based on mutations that activate MAPK kinase pathway (NRAS or PTPN11)

Table-1: Characteristics of the patients enrolled in analysis. AML risk was stratified by ELN risk stratification. ITD: Internal Tandem Duplication. TKD: Tyrosine Kinase Domains mutation. FLT3i: FLT3 inhibitor

Table-2: Types of combination therapies used with gilteritinib. FLAG: fludarabine, cytarabine and granulocyte colony-stimulating factor. CLIA: cladribine, idarubicin, and cytarabine. CLAG: cladribine, cytarabine and

granulocyte colony-stimulating factor. MEC: mitoxantrone, etoposide and cytarabine.

Supplementary material:

Figure -S1: Molecular landscape via Next Generation Sequencing (NGS) at diagnosis (A) and relapse (B).

Figure-S2: Type of FLT3 inhibitor (FLT3i) used prior to starting gilteritinib and type of response. Composite Complete Remission (CRc) is the combined rate of all CR, CRp and Cri. Mido: Midostaurin & Sora: Sorafenib

Patients' characteristics	All patients (n=113)
Age-year	
Mean (SD)	58.3 (15.4)
Range	18-92
Female- no.(%)	65 (57.5%)
Race	
White no.(%)	66 (58.4%)
African American no.(%)	11 (9.7%)
Other no.(%)	36 (31.9%)
AML risk	
Low/Intermediate no.(%)	73 (64.6%)
High no.(%)	36 (31.9%)
FLT3 mutation subtype	
ITD no.(%)	95 (84.1%)
TKD no.(%)	5 (4.4%)
Mixed	9 (8%)
Prior FLT3i	
Midostaurin no.(%)	65 (57.5%)
Sorafenib no.(%)	34 (30%)
Other FLT3i no.(%)	14 (12.5%)
Stem cell transplant (SCT)	
Before Gilteritinib no.(%)	49 (43.4%)
After Gilteritinib no.(%)	24 (21.2%).

Response to frontline FLT3i	
Relapse no.(%)	45 (39.8%)
Refractory no.(%)	68 (60.2%)
Use of Gilteritinib in clinical trial	
On a trial no.(%)	53 (46.9%)
Commercial use no.(%)	60 (53.1%)
Additional treatment to Gilteritinib	
Single agent no.(%)	71 (62.8%)
Combined with other agents no.(%)	42 (37.2%)

Type of Combination therapy	% of patients (n)	CRc rate in each group
In Combination High Intensity Chemotherapy (FLAG, CPX-351, CLIA, CLAG, MEC)	31% (13)	53.8% (7)
Hypomethylating agents (Decitabine or Azacitidine)	33% (14)	50% (7)
Single agent Venetoclax or Venetoclax with HMA	31% (13)	76.9% (10)
In Combination with IDH inhibitor	5% (2)	50% (1)

Figure-1a-d

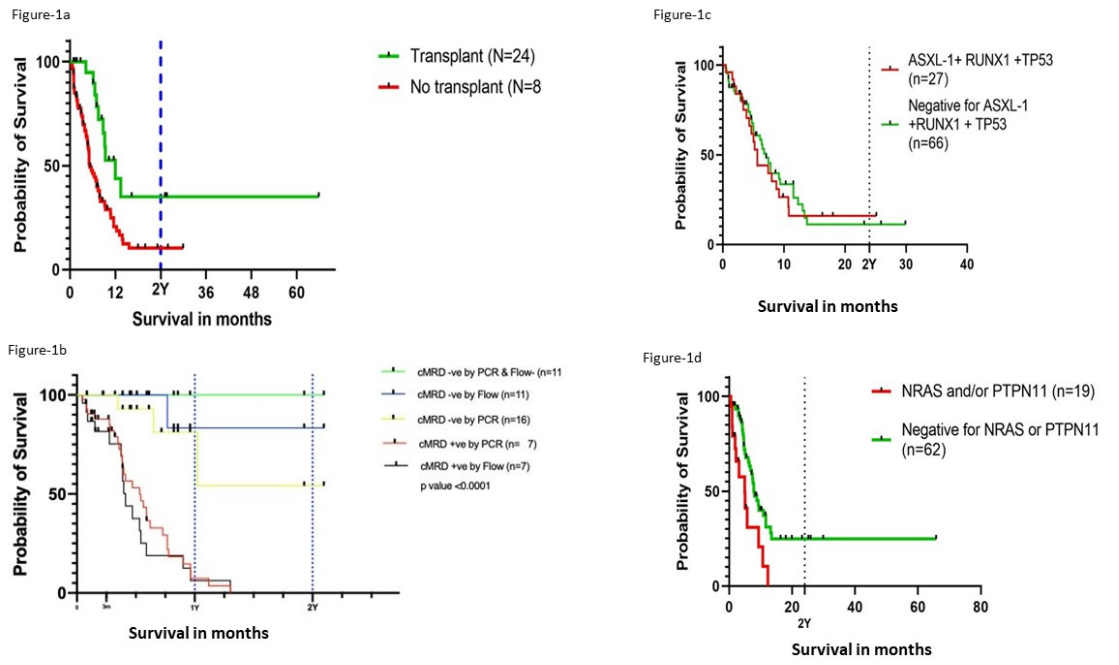


Figure-1a

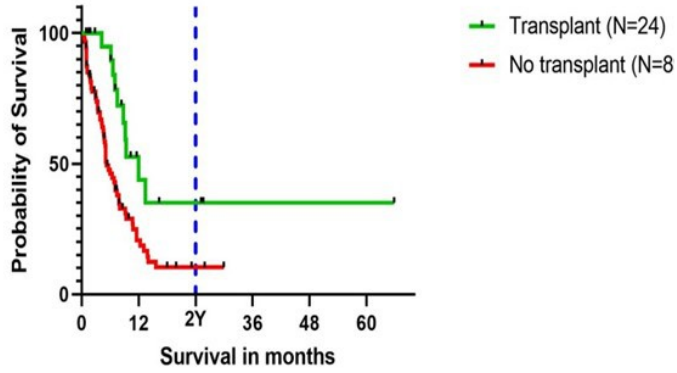


Figure-1c

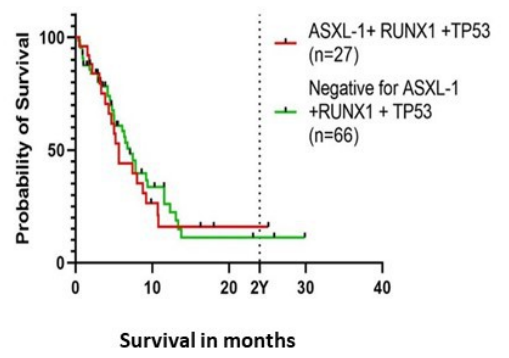


Figure-1b

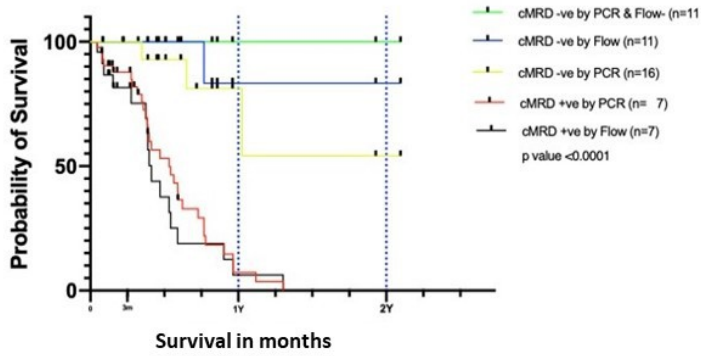


Figure-1d

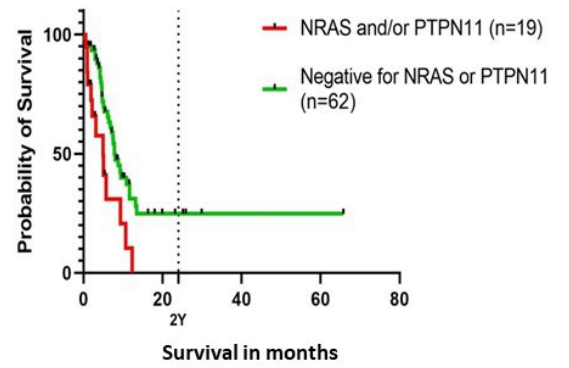


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