FLT3 inhibitors and hematopoietic cell transplantation prolong survival in patients with *FLT3*-ITD-positive AML

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Abstract
Acute myeloid leukemia (AML) is an aggressive hematological malignancy with genetic alterations. The FMS-like tyrosine kinase 3 (FLT3) gene is frequently mutated in adult *de novo* AML, with two types of mutations: internal tandem duplication (ITD) and point mutations in the tyrosine kinase domain. This study aimed to investigate the impact of FLT3 inhibitors and hematopoietic cell transplantation (HCT) on survival outcomes in patients with *FLT3*-ITD AML in a real-world setting. We retrospectively analyzed 139 patients with *FLT3*-ITD-positive AML between 2012 and 2021. The median age was 63 years. In the propensity score-matched cohort, FLT3 inhibitors improved overall survival (OS) compared with patients treated without FLT3 inhibitors (3-year OS: 33.7% vs. 25.8%, $p = 0.021$). Patients who underwent HCT had superior outcomes to those without HCT (3-year OS: 45.3% vs. 2.2%, $p < 0.0001$). The combination of FLT3 inhibitors and HCT resulted in the highest OS and relapse-free survival (RFS) rates (3-year OS: 62.4%; 3-year RFS: 44.4%). Disease status before transplantation did not predict the prognosis, but use of FLT3 inhibitors increased survival in patients without complete remission before HCT. These results demonstrate the clinical impact of FLT3 inhibitors on survival outcomes in patients with *FLT3*-ITD-positive AML, particularly when combined with HCT. FLT3 inhibitors can improve the prognosis of AML with *FLT3* mutations, especially in patients who undergo HCT.

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Keywords:
acute myeloid leukemia, *FLT3*-ITD, FLT3 inhibitor, hematopoietic cell transplantation

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Data availability

The datasets generated and/or analyzed during this study are available from the corresponding author upon reasonable request.

Conflict-of-interest disclosure

The authors declare that they have no conflicts of interest.

Ethical approval

All procedures involving human participants were in accordance with the ethical standards of institutional research committees, national guidelines, and with the Declaration of Helsinki. This study was approved by the institutional review board of Hokkaido University Hospital (#022-0085).

Authorship contributions

T. Matsukawa and T.K. designed this study. T. Matsukawa, M.O., T.K., M.K., D. Hidaka, S.O., A.M., A.S., T. Miyagishima, Y. Kakinoki, J.H., S.Y., M.Y., K.W., M.T., T. Ishihara, Y.H., A.F., T. Igarashi, T.S., S.I., R.K., H.S., K.F., J.I., Y. Kanisawa, S.H., T.E. and D. Hashimoto performed recruitment and treatment. T. Matsukawa, T.K., M.K., D. Hidaka, S.O., A.M., A.S., T. Miyagishima, Y. Kakinoki, J.H., S.Y., M.Y., K.W., M.T., T. Ishihara, Y.H., A.F., T. Igarashi, T.S., S.I., R.K., H.S., K.F., J.I., Y. Kanisawa, S.H., and T.E. collected the data. T. Matsukawa analyzed and interpreted data. T. Matsukawa and M.O. wrote the manuscript. T.T. supervised this study. All authors provided critique of the manuscript, contributed to the final version of the manuscript, and approved it for publication.

Introduction

Acute myeloid leukemia (AML) is an aggressive hematological malignancy characterized by karyotype abnormalities and gene mutations in hematopoietic stem cells and progenitor cells [1-3]. Genetic alterations in AML affect the prognosis, and FMS-like tyrosine kinase 3 (FLT3) mutations are frequently detected in patients with *de novo* AML. Internal tandem duplication (ITD) at the juxta membrane domain and point mutations in the tyrosine kinase domain (TKD) occur in approximately 25% and 5% of cases of adult AML, respectively [1]. The *FLT3* gene plays a pivotal role in the proliferation, differentiation, and survival of hematopoietic cells [4]. *FLT3* mutations constitutively activate multiple downstream signaling pathways, including phosphoinositide 3-kinase/Akt and Ras/mitogen-activated protein kinase, which promote cell growth and induce anti-apoptosis [4]. *FLT3*-ITD AML is associated with a poor prognosis, although emerging FLT3 inhibitors, such as midostaurin, quizartinib, and gilteritinib, have improved survival outcomes in these cases [5]. FLT3 inhibitors are classified as types 1 and 2. Type 1 inhibitors, such as gilteritinib, bind to FLT3 and inactivate FLT3 signaling pathways in both *FLT3*-ITD and *FLT3*-TKD, whereas type 2 inhibitors, such as quizartinib, show increased binding compared with type 1 inhibitors and decrease phosphorylation of FLT3 via *FLT3*-ITD [6,7]. FLT3 inhibitors are thus expected to increase survival and prevent relapse; however, their clinical impact in a real-world setting is still unclear. In Japan, the type 1 inhibitor, gilteritinib,

was approved in December 2018, followed by the type 2 inhibitor, quizartinib, in October 2019. This study aimed to evaluate the impact of FLT3 inhibitors on survival in patients with *FLT3*-ITD-positive AML in a real-world setting.

Patients and methods

Patient eligibility

We conducted a multicenter retrospective questionnaire-based study including patients with *FLT3*-ITD AML at 22 institutes in Hokkaido, Japan, from January 2012 to December 2021. Patients diagnosed with acute promyelocytic leukemia, patients who died within 14 days from diagnosis, and patients with only *FLT3*-TKD mutations were excluded. We defined patients who received FLT3 inhibitors for ≥14 days as "administration of FLT3 inhibitors" because a shorter usage of FLT3 inhibitors might not impact survival. *FLT3*-ITD was detected by companion diagnostics LeukoStrat CDx FLT3 or in-house analysis.

Endpoints

The primary endpoint in this study was overall survival (OS). The secondary endpoints were relapse-free survival (RFS) and rate of induction failure. OS was defined from the day of diagnosis with AML to death. RFS was defined from the day of achievement of complete

remission (CR) or CR with incomplete recovery to death or hematological relapse [8].

Propensity score (PS) matching

To avoid potential confounding in patient characteristics, we calculated the propensity scores (PS), and caliper-matched cases with or without FLT3 inhibitors in a 1:1 ratio using the nearest-neighbor matching method with multivariable logistic regression analysis. The covariates were sex (female or male), age (≥65 or <65 years), Eastern Cooperative Oncology Group (ECOG) performance status (0–2 or ≥3), and patients who underwent HCT (yes or no), as factors that might affect survival outcomes.

Statistical analysis

The patients' characteristics were tested by χ^2 and Fisher's exact tests for categorical data or the Mann–Whitney U test for quantitative data. OS and RFS were calculated using the Kaplan–Meier method. All statistical tests were two-sided, and a p value < 0.05 was considered significant. All statistical analyses were performed using the EZR software package in R commander for macOS version 1.61 (Saitama Medical Center, Jichi Medical University; http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html).

Results

Patient characteristics

A total of 171 patients were registered, of whom 17 patients with acute promyelocytic leukemia, 11 patients who died within 14 days from diagnosis, and 4 patients with only *FLT3*-TKD mutations were excluded. A total of 139 patients met the eligibility criteria. (Table 1) The median age was 63 years (range 7–95 years) and there were 56 (40.3%) women and 83 (59.7%) men (Supplementary Fig. 1). The median ECOG performance status was 1 (range, 0–4), the median white blood cell count (WBC) at diagnosis was 52,630/μL (range, 680–496,000), and the median serum lactate dehydrogenase (LDH) level was 544.5 U/L (range, 20–11,937). One hundred patients (71.9%) received intensive chemotherapy (idarubicin + cytarabine, n = 76; daunorubicin + cytarabine, n = 24). The rest of the patients received non-intensive treatment, including a low dose cytarabine regimen (n = 18), azacytidine alone (n = 6), venetoclax + azacytidine (n = 3), hydroxyurea (n = 6), or best supportive care (n = 6). Among the 100 patients who underwent intensive chemotherapy, 67 (67.0%) achieved CR after first induction therapy. Among all 139 eligible cases, a normal karyotype was detected in 104 (74.8%). HCT was conducted in 65 cases (46.8%), with 52 and 13 patients receiving myeloablative conditioning and reduced-intensity conditioning regimens, respectively. *FLT3*-ITD mutations alone occurred in 134 (96.4%) patients and *FLT3*-ITD plus *FLT*-TKD mutations occurred in 5 patients (3.6%).

FLT3 inhibitors were administered in 59 cases (42.4%) based on the study criteria for the indication of primary refractory/relapse (n = 40) or maintenance (n = 19). There was no significant difference in patient age between patients treated with and without FLT3 inhibitors (median 65 years, range 7–90; median 60.5 years, range 9–95, respectively) (p = 0.76). There were also no significant differences between the two groups in terms of sex (male: 59.3% vs. 60.0%, p = 1), median WBC at diagnosis (61,280/μL vs. 40,520/μL, p = 0.55), median serum LDH level (585 U/L vs. 522 U/L, p = 0.64), CR after one course of induction therapy (50.8% vs. 46.2%, p = 0.72), cases who underwent HCT (47.5% vs. 46.3%, p = 1), and *FLT3*-ITD alone (94.9% vs. 97.5%, p = 0.73) (Supplementary Table 1).

We next compared the patient characteristics in the PS-matched cohort (n = 56 per group). There were no significant differences between the two groups in terms of age (median 64 years vs. 63 years, p = 0.63), sex (male: 57.2% vs. 55.4%, p = 1), ECOG performance status (1 vs. 1, p = 1), median WBC (62,150/μL vs. 51,770/μL, p = 0.56), serum LDH level (609.5 U/L vs. 524 U/L, p = 0.49), number of cases with intensive chemotherapy (42 vs. 38 cases, p = 0.59), CR rate after one course of induction therapy (64.3% vs. 68.4%, p = 0.70), patients who underwent HCT (50% vs. 50%, p = 1), and *FLT3*-ITD alone (98.2% vs. 96.4%, p = 1) (Table 2). Among patients who underwent HCT, FLT3 inhibitors were administered only before HCT (n = 5, 17.9%), only after HCT (n = 9, 32.1%), both before and after HCT for maintenance (n = 10, 35.7%), and

193 at relapse after HCT (n = 4, 14.3%). The FLT3 inhibitor was gilteritinib alone in 44, quizartinib
194 alone in one, sorafenib alone in one, and sequential gilteritinib and quizartinib in 10 patients.
195 FLT3 inhibitors were administered without transplantation for relapsed or refractory disease, not
196 for induction, because upfront FLT3 inhibitors were only approved as induction therapy in Japan
197 in May 2023, after this study period.
198
199 **Survival outcomes**
200 The 3-year OS and RFS in the whole study cohort were 27.4% (95% confidence interval
201 [CI], 19.6%–35.7%) and 32.1% (95% CI, 21.0%–43.7%), respectively (Fig. 1a, b). We compared
202 survival according to age: <18 years, adult/young adult (18–40 years), 41–70 years, and >70 years.
203 There were only five patients aged <18 years, but these limited cases showed favorable OS (3-
204 year OS: 60% [95% CI, 12.6%–88.2%]). Patients in the 18–40 and 41–70 year groups
205 demonstrated 3-year OS rates of 51.8% (95% CI, 26.2%–72.4%) and 30.2% (95% CI, 19.5%–
206 41.6%), respectively. However, patients aged >70 years showed poor OS (4.7% [95% CI, 0.4%–
207 18.6%]) (Fig. 1c). The 3-year RFS rates in patients aged <18, 18–40, 41–70, and >70 years were
208 66.7% (95% CI, 5.4%–94.5%), 42.9% (95% CI, 9.8%–73.4%), 28.9% (95% CI, 16.6%–42.4%),
209 and not reached, respectively (Fig. 1d). The use of FLT3 inhibitors divided the prognosis, with
210 patients receiving FLT3 inhibitors having better OS than those without FLT inhibitors (3-year OS:

32.4% vs. 23.4%, p = 0.0068) (Supplementary Fig. 2a). RFS in the group treated with FLT3 inhibitors in the whole cohort was similar to the group without FLT3 inhibitors (3-year RFS: 29.0% vs. 34.4%, p = 0.11) (Supplementary Fig. 2b). OS and RFS were improved in patients with HCT compared with those without HCT (3-year OS: 45.3% vs. 2.2%, p < 0.0001; 3-year RFS: 43.4% vs. 12.1%, p = 0.023) (Supplementary Fig. 2c, d). OS was highest among patients treated with FLT3 inhibitors and HCT compared with the other groups (p < 0.0001) (Supplementary Fig. 2e, f). The hazard ratios for OS according to use of FLT3 inhibitors and HCT in all eligible cases were 0.57 (95% CI, 0.38–0.86, p = 0.0076) and 0.31 (95% CI, 0.21–0.48, p < 0.0001), respectively.

The 3-year OS and RFS rates in the PS-matched cohort were 29.7% (95% CI, 20.9%–39.1%) and 31.8% (95% CI, 19.6%–44.7%), respectively (Fig. 2a, b). FLT3 inhibitors improved OS in the PS-matched cohort compared with the group without FLT3 inhibitors (3-year OS: 33.7% vs. 25.8%, p = 0.021), but had no effect on RFS (27.6% vs. 35.4%, p = 0.059) (Fig. 2c, d). HCT improved OS and RFS in the PS-matched cohort compared with patients without HCT (3-year OS: 47.9% vs. 6.8%, p < 0.0001; 3-year RFS: 42.7% vs. not reached, p = 0.0080, respectively) (Fig. 2e, f). In the PS-matched cohort, FLT3 inhibitors plus HCT also improved OS (3-year OS: FLT3 inhibitors + HCT, 62.4%; HCT alone, 34.8%; FLT inhibitors alone, not reached; no FLT3 inhibitors or HCT, 17.4%; p < 0.0001) and RFS (3-year RFS: FLT3 inhibitors + HCT, 44.4%; HCT alone, 41.2%; FLT inhibitors alone, not reached; no FLT3 inhibitors or HCT, not

reached; p = 0.019) (Fig. 2g, h). Among patients who underwent HCT, hematological CR did not affect survival compared with non-CR (3-year OS: 50.5% vs. 40.0%, p = 0.44) (Supplementary Fig. 3a). Patients without CR receiving FLT3 inhibitors demonstrated superior survival outcomes compared with patients without CR and without FLT3 inhibitors (3-year OS: 62.5% vs. 16.7%, p = 0.022), (Supplementary Fig. 3b), although the comparison was limited by the small number of cases. The use of FLT inhibitors did not affect survival among patients with CR before HCT (3-year OS: with inhibitors 62.9% vs. without inhibitors 40.0%, p = 0.15).

Discussion

We retrospectively analyzed patients with *FLT3*-mutated AML in a regional multicenter cohort in Japan. The results showed that FLT3 inhibitors and HCT improved survival outcomes among patients with *FLT3*-ITD AML, while the combination of FLT3 inhibitors and HCT led to superior outcomes compared with the other treatment groups. FLT3 inhibitor administration improved outcomes among patients without HCT, but HCT had a more significant impact than FLT3 inhibitors. Among patients undergoing HCT, FLT3 inhibitors had no impact in patients with hematological CR before HCT, but improved survival in patients without CR before HCT, compared with patients without FLT3 inhibitors. Gilteritinib was approved for relapsed/refractory *FLT3*-ITD/-TKD mutated AML and quizartinib was approved for newly diagnosed and

247 relapsed/refractory *FLT3*-ITD AML in Japan, but quizartinib was not approved for newly
248 diagnosed AML during the study period. Upfront FLT3 inhibitors combined with conventional
249 7+3 induction might enhance the impact of FLT3 inhibitors.

250 Midostaurin, a first-generation type 1 tyrosine kinase inhibitor for *FLT3*-mutated AML,
251 inhibits multi-kinases such as FLT3, KIT, and platelet-derived growth factor receptor. In the
252 randomized phase 3 RATIFY trial, midostaurin with conventional 7+3 induction therapy,
253 improved OS compared with conventional chemotherapy alone in patients with newly diagnosed
254 *FLT3*-mutated AML, but had no additional effect in patients aged ≥60 years [9]. First-generation
255 FLT3 inhibitors such as midostaurin have poor specificity for FLT3 and many off-target effects,
256 leading to side effects, including hematological adverse events [6]. Most cases (96.2%) in the
257 current PS-matched cohort who were treated with FLT3 inhibitors initially selected gilteritinib,
258 because this was the first-in-class agent to be approved in Japan. Gilteritinib is a second-
259 generation type 1 FLT3 inhibitor with a high affinity for mutated FLT3 receptors that inhibits the
260 phosphorylation of FLT3 and AXL. The randomized phase 3 ADMIRAL trial showed that
261 gilteritinib prolonged survival in patients with relapsed or refractory *FLT3*-mutated AML and
262 induced a high incidence of CR compared with patients who received conventional salvage
263 chemotherapy [10]. In the current study, disease status before transplantation did not affect OS,
264 but the use of FLT3 inhibitors improved survival in patients without CR undergoing HCT. Low-

dose gilteritinib after HCT also improved outcomes in initiating early post-transplantation as maintenance therapy [11]. In the ADMIRAL trial, patients who underwent HCT showed comparable outcomes between the pretransplant gilteritinib and salvage chemotherapy arms, but patients who resumed gilteritinib after HCT had better outcomes than those who did not resume gilteritinib in the gilteritinib arm [12]. Moreover, Levis et al. reported that patients with *FLT3*-ITD with hematological CR of 1×10^{-6} or greater for *FLT3*-ITD had poor OS and RFS without gilteritinib, but gilteritinib combined with HCT increased survival among these patients [13]. Maintenance therapy with FLT3 inhibitors after transplantation might prevent relapse and improve prognosis, but patients eligible for maintenance therapy after HCT need to be selected in terms of disease status pre- or post-transplantation.

The second-generation type 2 FLT3 inhibitor, quizartinib, selectively inhibits FLT3 but has lower affinity to KIT, RET, colony-stimulating factor-1 receptor, and platelet-derived growth factor receptor [7]. In a phase 1 clinical trial of post-transplant maintenance therapy, four (31%) patients survived >2 years; however, the sample size was very small, and no large randomized trials of quizartinib have been conducted for *FLT3*-ITD AML after HCT [14]. A study using real-world data for 1208 patients with *FLT3*-mutated AML from eight countries showed that post-transplant administration of FLT3 inhibitors (n = 219, 18.1%), including midostaurin (n = 140, 63.9%), sorafenib (n = 58, 26.5%), gilteritinib (n = 14, 6.4%), and quizartinib (n = 9, 4.1%), or

other maintenance therapy (n = 224, 18.5%) improved survival [15]. In a randomized phase 3 trial, quizartinib in combination with intensive chemotherapy, improved OS, especially in cases with a higher *FLT3*-ITD variant allelic frequency [16]; however, quizartinib with conventional chemotherapy still failed to improve OS in patients aged ≥60 years, and the study did not include patients who received FLT3 inhibitors as induction therapy. FLT3 inhibitors improved survival in patients with relapsed or refractory *FLT3*-ITD AML without HCT in the current study, as reported in previous phase 3 studies of gilteritinib and quizartinib [10,17].

The current study had several limitations. First, the study retrospectively analyzed a small number of cases. Second, genetic alterations other than *FLT3* mutations could not be assessed because a comprehensive panel for genetic mutations has not been approved in Japan. Third, FLT3 inhibitors were administered for many purposes in the patients who underwent HCT, including maintenance before transplantation and relapse after transplantation. Finally, the disease status before HCT was only assessed by morphological evaluation.

In conclusion, this retrospective multicenter study revealed that FLT3 inhibitors and HCT improved survival in patients with *FLT3*-ITD AML in a real-world setting. HCT had a more significant impact on survival among these patients, and intensive chemotherapy plus FLT3 inhibitors with HCT for fit patients with *FLT3*-ITD AML might lead to better outcomes. Further studies are needed to determine which patients are suitable for FLT3 inhibitors as maintenance

301 therapy after HCT.

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303

304 **Figure legends**

305 **Fig. 1** Overall survival (OS) and relapse-free survival (RFS) in the whole study cohort. A, B. OS

306 and RFS. C, D. OS and RFS according to age group: <18, 18–40, 41–70, and >70 years

307

308 **Fig. 2** Overall survival (OS) and relapse-free survival (RFS) in propensity score (PS)-matched

309 patients. A, B. OS and RFS in the whole PS-matched cohort. FLT3 inhibitors (C, D) hematopoietic

310 cell transplantation (HCT) (E, F), and FLT3 inhibitors plus HCT (G, H) improved survival

311 outcomes

312

313 **Supplementary Fig. 1** Age distribution of total cohort (n = 139) showing sex (A) and patients

314 who underwent hematopoietic cell transplantation (HCT) (B)

315

316 **Supplementary Fig. 2** Overall survival (OS) and relapse-free survival (RFS) in all patients. FLT3

317 inhibitors (A, B), hematopoietic cell transplantation (HCT) (C, D), and FLT3 inhibitors plus HCT

318 (E, F) improved survival outcomes

Supplementary Fig. 3 Overall survival (OS) in propensity score-matched patients with hematopoietic cell transplantation (HCT). A. Disease status before hematopoietic complete remission (CR) did not affect OS compared with patients without CR ($p = 0.44$). B. FLT3 inhibitors improved OS in patients without CR

References

1. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, Gundem G, Van Loo P, Martincorena I, Ganly P, Mudie L, McLaren S, O'Meara S, Raine K, Jones DR, Teague JW, Butler AP, Greaves MF, Ganser A, Dohner K, Schlenk RF, Dohner H, Campbell PJ (2016) Genomic Classification and Prognosis in Acute Myeloid Leukemia. The New England journal of medicine 374 (23):2209-2221. doi:10.1056/NEJMoa1516192

2. Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, Hoadley K, Triche TJ, Jr., Laird PW, Baty JD, Fulton LL, Fulton R, Heath SE, Kalicki-Veizer J, Kandoth C, Klco JM, Koboldt DC, Kanchi KL, Kulkarni S, Lamprecht TL, Larson DE, Lin L, Lu C, McLellan MD, McMichael JF, Payton J, Schmidt H, Spencer DH, Tomasson MH, Wallis JW, Wartman LD, Watson MA, Welch

337 J, Wendl MC, Ally A, Balasundaram M, Birol I, Butterfield Y, Chiu R, Chu A, Chuah E, Chun HJ,
338 Corbett R, Dhalla N, Guin R, He A, Hirst C, Hirst M, Holt RA, Jones S, Karsan A, Lee D, Li HI,
339 Marra MA, Mayo M, Moore RA, Mungall K, Parker J, Pleasance E, Plettner P, Schein J, Stoll D,
340 Swanson L, Tam A, Thiessen N, Varhol R, Wye N, Zhao Y, Gabriel S, Getz G, Sougnez C, Zou L,
341 Leiserson MD, Vandin F, Wu HT, Applebaum F, Baylin SB, Akbani R, Broom BM, Chen K, Motter
342 TC, Nguyen K, Weinstein JN, Zhang N, Ferguson ML, Adams C, Black A, Bowen J, Gastier-Foster
343 J, Grossman T, Lichtenberg T, Wise L, Davidsen T, Demchok JA, Shaw KR, Sheth M, Sofia HJ,
344 Yang L, Downing JR, Eley G (2013) Genomic and epigenomic landscapes of adult de novo acute
345 myeloid leukemia. The New England journal of medicine 368 (22):2059-2074.
346 doi:10.1056/NEJMoa1301689

347 3. Matsukawa T, Aplan PD (2020) Clinical and molecular consequences of fusion genes in myeloid
348 malignancies. Stem Cells 38 (11):1366-1374. doi:10.1002/stem.3263

349 4. Gilliland DG, Griffin JD (2002) The roles of FLT3 in hematopoiesis and leukemia. Blood 100
350 (5):1532-1542. doi:10.1182/blood-2002-02-0492

351 5. Daver N, Schlenk RF, Russell NH, Levis MJ (2019) Targeting FLT3 mutations in AML: review
352 of current knowledge and evidence. Leukemia 33 (2):299-312. doi:10.1038/s41375-018-0357-9

353 6. Kiyoi H, Kawashima N, Ishikawa Y (2020) FLT3 mutations in acute myeloid leukemia:
354 Therapeutic paradigm beyond inhibitor development. Cancer science 111 (2):312-322.

355 doi:10.1111/cas.14274

356 7. Zarrinkar PP, Gunawardane RN, Cramer MD, Gardner MF, Brigham D, Belli B, Karaman MW,

357 Pratz KW, Pallares G, Chao Q, Sprankle KG, Patel HK, Levis M, Armstrong RC, James J, Bhagwat

358 SS (2009) AC220 is a uniquely potent and selective inhibitor of FLT3 for the treatment of acute

359 myeloid leukemia (AML). Blood 114 (14):2984-2992. doi:10.1182/blood-2009-05-222034

360 8. Döhner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, Dombret H, Fenaux

361 P, Grimwade D, Larson RA, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz MA,

362 Sierra J, Tallman MS, Löwenberg B, Bloomfield CD (2010) Diagnosis and management of acute

363 myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the

364 European LeukemiaNet. Blood 115 (3):453-474. doi:10.1182/blood-2009-07-235358

365 9. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, Thiede C, Prior

366 TW, Döhner K, Marcucci G, Lo-Coco F, Klisovic RB, Wei A, Sierra J, Sanz MA, Brandwein JM,

367 de Witte T, Niederwieser D, Appelbaum FR, Medeiros BC, Tallman MS, Krauter J, Schlenk RF,

368 Ganser A, Serve H, Ehninger G, Amadori S, Larson RA, Döhner H (2017) Midostaurin plus

369 Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. The New England journal of

370 medicine 377 (5):454-464. doi:10.1056/NEJMoa1614359

371 10. Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, Montesinos P, Baer MR,

372 Larson RA, Ustun C, Fabbiano F, Erba HP, Di Stasi A, Stuart R, Olin R, Kasner M, Ciceri F, Chou

WC, Podoltsev N, Recher C, Yokoyama H, Hosono N, Yoon SS, Lee JH, Pardee T, Fathi AT, Liu C, Hasabou N, Liu X, Bahceci E, Levis MJ (2019) Gilteritinib or Chemotherapy for Relapsed or Refractory FLT3-Mutated AML. The New England journal of medicine 381 (18):1728-1740. doi:10.1056/NEJMoa1902688

11. Terao T, Matsuoka KI, Ueda H, Matsumura A, Matsubara C, Kondo K, Kondo T, Fujiwara H, Asada N, Ennishi D, Nishimori H, Fujii K, Fujii N, Maeda Y (2023) Early initiation of low-dose gilteritinib maintenance improves posttransplant outcomes in patients with R/R FLT3mut AML. Blood Adv 7 (5):681-686. doi:10.1182/bloodadvances.2022008991

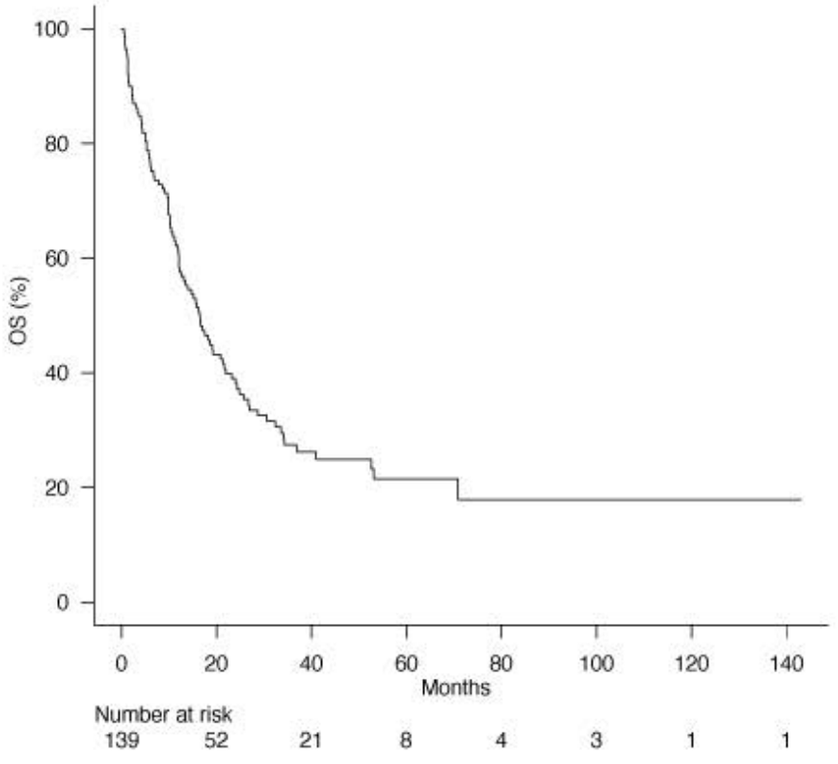
12. Perl AE, Larson RA, Podoltsev NA, Strickland S, Wang ES, Atallah E, Schiller GJ, Martinelli G, Neubauer A, Sierra J, Montesinos P, Recher C, Yoon SS, Maeda Y, Hosono N, Onozawa M, Kato T, Kim HJ, Hasabou N, Nuthethi R, Tiu R, Levis MJ (2023) Outcomes in Patients with FLT3-Mutated Relapsed/ Refractory Acute Myelogenous Leukemia Who Underwent Transplantation in the Phase 3 ADMIRAL Trial of Gilteritinib versus Salvage Chemotherapy. Transplant Cell Ther 29 (4):265.e261-265.e210. doi:10.1016/j.jtct.2022.12.006

13. Levis MJ, Hamadani M, Logan B, Jones RJ, Singh AK, Litzow M, Wingard JR, Papadopoulos EB, Perl AE, Soiffer RJ, Ustun C, Ueda Oshima M, Uy GL, Waller EK, Vasu S, Solh M, Mishra A, Muffly L, Kim HJ, Mikesch JH, Najima Y, Onozawa M, Thomson K, Nagler A, Wei AH, Marcucci G, Geller NL, Hasabou N, Delgado D, Rosales M, Hill J, Gill SC, Nuthethi R, King D, Wittsack H,

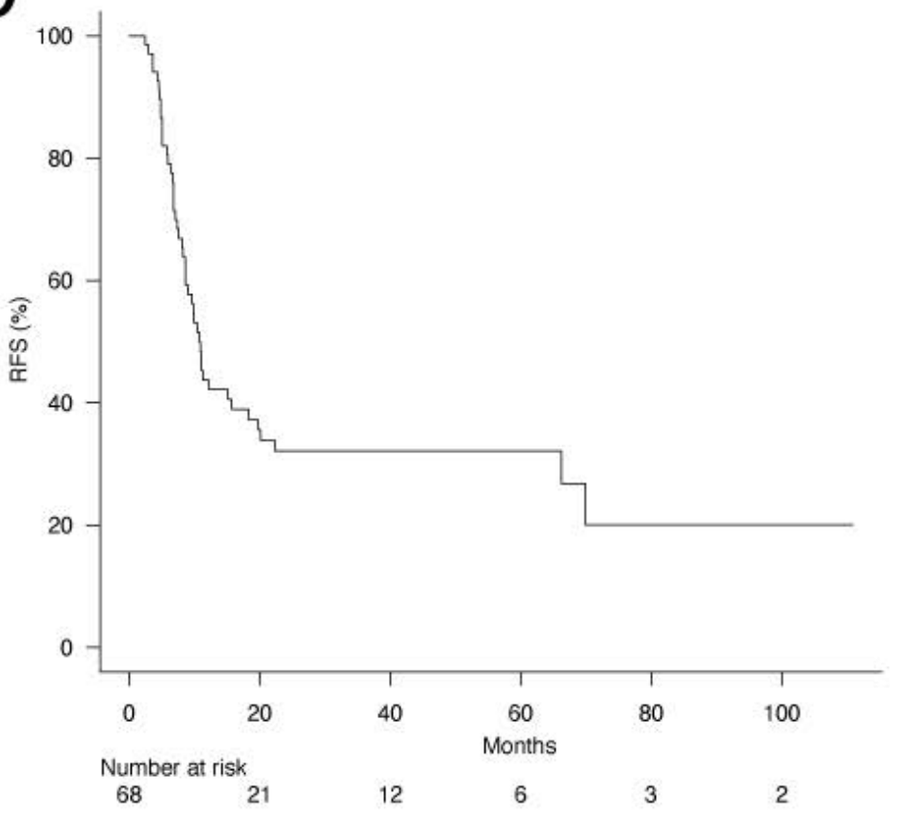
391 Mendizabal A, Devine SM, Horowitz MM, Chen YB (2024) Gilteritinib as Post-Transplant
392 Maintenance for AML With Internal Tandem Duplication Mutation of FLT3. Journal of clinical
393 oncology : official journal of the American Society of Clinical Oncology 42 (15):1766-1775.
394 doi:10.1200/jco.23.02474
395 14. Sandmaier BM, Khaled S, Oran B, Gammon G, Trone D, Frankfurt O (2018) Results of a
396 phase 1 study of quizartinib as maintenance therapy in subjects with acute myeloid leukemia in
397 remission following allogeneic hematopoietic stem cell transplant. Am J Hematol 93 (2):222-231.
398 doi:10.1002/ajh.24959
399 15. Griffin JD, Song Y, Yang H, Freimark J, Shah MV (2021) Post-transplant maintenance therapy
400 in patients with FLT3-mutated acute myeloid leukemia: Real-world treatment patterns and
401 outcomes. European journal of haematology 107 (5):553-565. doi:10.1111/ejh.13692
402 16. Erba HP, Montesinos P, Kim HJ, Patkowska E, Vrhovac R, Žák P, Wang PN, Mitov T, Hanyok
403 J, Kamel YM, Rohrbach JEC, Liu L, Benzohra A, Lesegretain A, Cortes J, Perl AE, Sekeres MA,
404 Dombret H, Amadori S, Wang J, Levis MJ, Schlenk RF (2023) Quizartinib plus chemotherapy in
405 newly diagnosed patients with FLT3-internal-tandem-duplication-positive acute myeloid
406 leukaemia (QuANTUM-First): a randomised, double-blind, placebo-controlled, phase 3 trial.
407 Lancet 401 (10388):1571-1583. doi:10.1016/s0140-6736(23)00464-6
408 17. Cortes JE, Khaled S, Martinelli G, Perl AE, Ganguly S, Russell N, Krämer A, Dombret H,

409 Hogge D, Jonas BA, Leung AY, Mehta P, Montesinos P, Radsak M, Sica S, Arunachalam M,
410 Holmes M, Kobayashi K, Namuyinga R, Ge N, Yver A, Zhang Y, Levis MJ (2019) Quizartinib
411 versus salvage chemotherapy in relapsed or refractory FLT3-ITD acute myeloid leukaemia
412 (QuANTUM-R): a multicentre, randomised, controlled, open-label, phase 3 trial. Lancet Oncol
413 20 (7):984-997. doi:10.1016/s1470-2045(19)30150-0
414

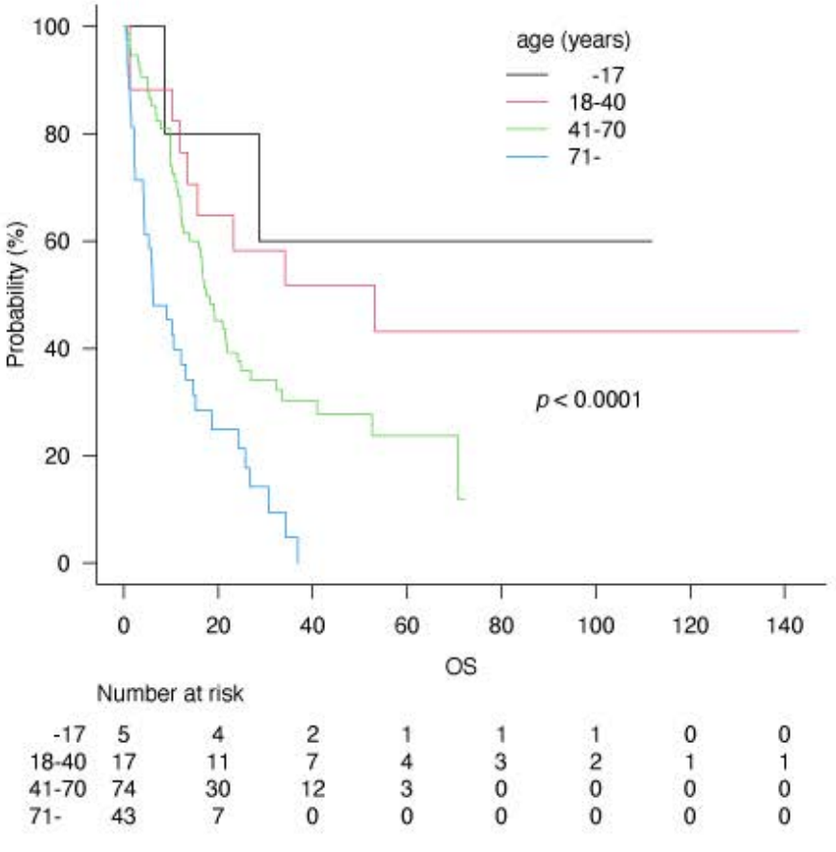
a


OS (%)
100
80
60
40
20
0
0 20 40 60 80 100 120 140
Months
Number at risk
139 52 21 8 4 3 1 1

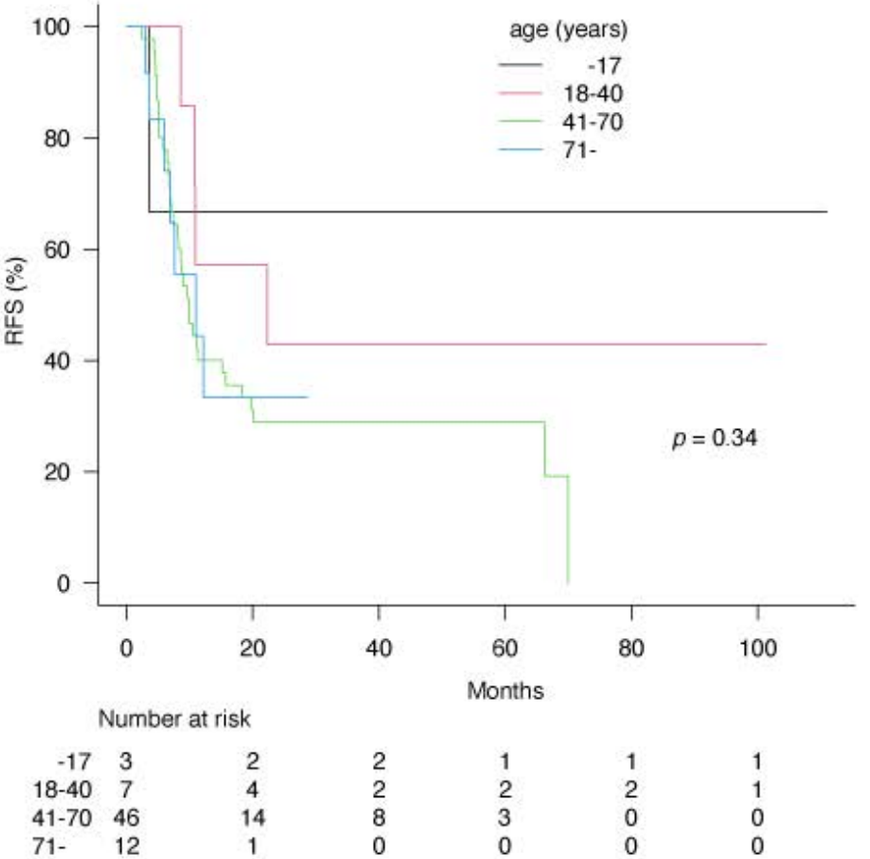
b

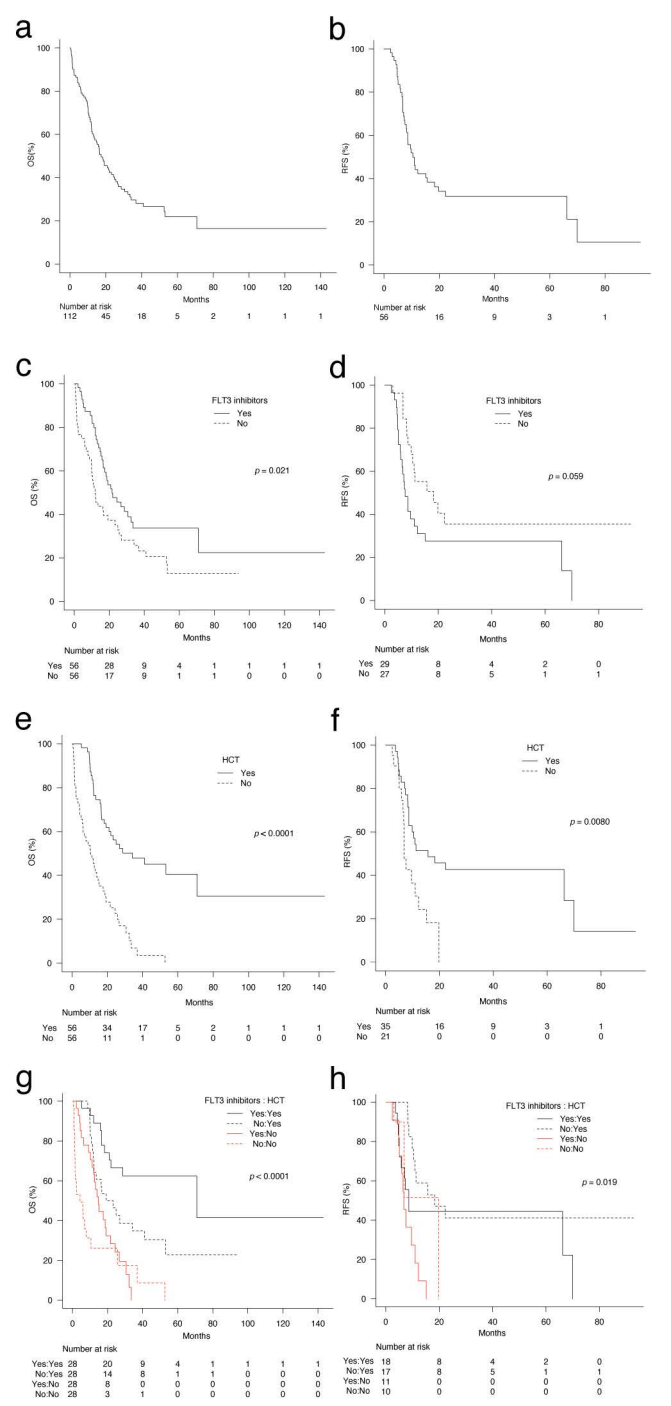

RFS (%)
100
80
60
40
20
0
0 20 40 60 80 100
Months
Number at risk
68 21 12 6 3 2

c


Probability (%)
100
80
60
40
20
0
age (years)
-17
18-40
41-70
71-
p < 0.0001
0 20 40 60 80 100 120 140
OS
Number at risk
-17 5 4 2 1 1 1 0 0
18-40 17 11 7 4 3 2 1 1
41-70 74 30 12 3 0 0 0 0
71- 43 7 0 0 0 0 0 0

d


RFS (%)
100
80
60
40
20
0
age (years)
-17
18-40
41-70
71-
p = 0.34
0 20 40 60 80 100
Months
Number at risk
-17 3 2 2 1 1 1
18-40 7 4 2 2 2 1
41-70 46 14 8 3 0 0
71- 12 1 0 0 0 0


a
OS(%)
Months
Number at risk
112 45 18 5 2 1 1 1
b
RFS (%)
Months
Number at risk
56 16 9 3 1
c
FLT3 inhibitors
Yes
No
p = 0.021
OS (%)
Months
Number at risk
Yes 56 28 9 4 1 1 1 1
No 56 17 9 1 1 0 0 0
d
FLT3 inhibitors
Yes
No
p = 0.059
RFS (%)
Months
Number at risk
Yes 29 8 4 2 0
No 27 8 5 1 1
e
HCT
Yes
No
p < 0.0001
OS (%)
Months
Number at risk
Yes 56 34 17 5 2 1 1 1
No 56 11 1 0 0 0 0 0
f
HCT
Yes
No
p = 0.0080
RFS (%)
Months
Number at risk
Yes 35 16 9 3 1
No 21 0 0 0 0
g
FLT3 inhibitors : HCT
Yes:Yes
No:Yes
Yes:No
No:No
p < 0.0001
OS (%)
Months
Number at risk
Yes:Yes 28 20 9 4 1 1 1 1
No:Yes 28 14 8 1 1 0 0 0
Yes:No 28 8 0 0 0 0 0 0
No:No 28 3 1 0 0 0 0 0
h
FLT3 inhibitors : HCT
Yes:Yes
No:Yes
Yes:No
No:No
p = 0.019
RFS (%)
Months
Number at risk
Yes:Yes 18 8 4 2 0
No:Yes 17 8 5 1 1
Yes:No 11 0 0 0 0
No:No 10 0 0 0 0