QuANTUM-First Trial: *FMS*-Like Tyrosine Kinase 3-Internal Tandem Duplication (*FLT3*-ITD)–Specific Measurable Residual Disease (MRD) Clearance Assessed Through Induction and Consolidation Is Associated With Improved Overall Survival in Newly Diagnosed *FLT3*-ITD+ AML Patients

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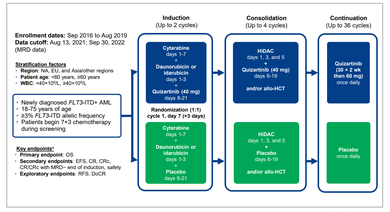
INTRODUCTION

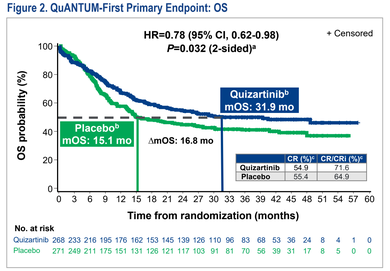
- *FLT3*-ITD mutations are the most common type of FLT3-activating mutation in acute myeloid leukemia (AML) and that they have a negative prognostic impact[1-3]
- There are currently 3 United States Food and Drug Administration– and European Medicines Agency–approved FLT3 inhibitors (midostaurin,[4] gilteritinib,[5] and quizartinib[6])
- Midostaurin and gilteritinib are type 1 inhibitors, essentially ATP-mimetics that inhibit both ITD and tyrosine kinase domain (TKD) mutations[4,5]
- Quizartinib is a type 2 inhibitor, more potent and selective than either midostaurin or gilteritinib, specifically active against the ITD mutations[1,3]
- QuANTUM-First (NCT02668653, phase 3; **Figure 1**) showed that in newly diagnosed patients with *FLT3*-ITD+ AML, adding the FLT3 inhibitor quizartinib to standard chemotherapy with or without allogeneic hematopoietic cell transplantation, followed by quizartinib or placebo monotherapy for up to 3 years, decreased the relative risk of death by 22.4% versus placebo with a generally manageable safety profile (**Figure 2**)[6]

Figure 1. QuANTUM-First Phase 3 Trial



[a]A hierarchical testing procedure was used to test the primary endpoint of OS, followed by EFS, CR, CRc, CR with *FLT3*-ITD MRD negativity, and CRc with *FLT3*-ITD MRD negativity.
CR, complete remission; CRc, composite complete remission; DoCR, duration of complete remission; EFS, event-free survival; EU, Europe; allo-HCT, allogeneic hematopoietic cell transplantation; *FLT3*-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; HiDAC, high-dose cytarabine; MRD, measurable residual disease; NA, North America; OS, overall survival; RFS, relapse-free survival; WBC, white blood cell.

Figure 2. QuANTUM-First Primary Endpoint: OS



	CR (%)[c]	CR/CRi (%)[c]
Quizartinib	54.9	71.6
Placebo	55.4	64.9

[a]*P* value was calculated using a stratified log-rank test. [b]Median follow-up time for both arms was 39.2 months. [c]CR/CRi per IRC after 1-2 courses of induction. CR, complete remission; CRi, complete remission with incomplete neutrophil or platelet recovery; HR, hazard ratio; mOS, median overall survival; OS, overall survival.

- Measurable residual disease is a well-established prognostic factor impacting relapse and survival in all stages of AML[7-9]
- Based on a retrospective *FLT3*-ITD MRD analysis by PCR-NGS conducted on 161 newly diagnosed patients with *FLT3*-ITD+ AML enrolled in phase 3 HOVON-SAKK clinical trials, MRD after 2 cycles of intensive chemotherapy was associated with increased relapse risk and reduced overall survival (OS) (**Table 1**)[10]

Table 1. Correlation Between MRD After Intensive Chemotherapy and Outcomes[10]

	FLT3-ITD MRD+ (n=47)	*FLT3*-ITD MRD– (n=114)	HR (95% CI)	*P* value
4-year CIR	75%	33%	3.70 (2.31-5.94)	*P*<0.001
4-year OS	31%	57%	2.47 (1.59-3.84)	*P*<0.001

CIR, cumulative incidence of relapse; *FLT3*-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; HR, hazard ratio; MRD, measurable residual disease; OS, overall survival.

- It is known that distal ITD insertion sites are associated with long ITD insert size[11]
  - In the RATIFY trial, patients with the most distal ITD insertion site (tyrosine kinase domain-1) had a significantly inferior OS compared with patients with more proximal insertion sites (juxtamembrane domain; **Table 2**)[12]
  - The negative impact conferred by the most distal ITD insertion sites was not improved by midostaurin treatment[12]
- Retrospective UK cooperative group data suggested that multiple *FLT3*-ITDs worsen survival, but follow-up studies did not confirm this[13,14]
  - A limitation of these studies was the low-sensitivity PCR assay used, which cannot not detect low-level variant allele frequency (VAF) *FLT3*-ITDs easily seen by PCR-NGS

Table 2. ITD Insertion Site Analysis in RATIFY[12]

	TKD1 sole (n=84)		JMD sole (n=251)		*P* value
4-year OS	29%		44%		*P*=0.032
	TKD1 sole (n=84)		JMD sole (n=251)		
	Midostaurin (n=55)	Placebo (n=29)	Midostaurin (n=119)	Placebo (n=132)	
4-year OS	32%	26%	48%	40%	
P value	*P*=0.256		*P*=0.047		

ITD, internal tandem duplication; JMD, juxtamembrane domain; OS, overall survival; TKD1, tyrosine kinase domain-1.

PURPOSE

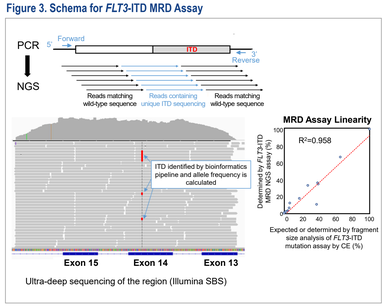
- Using samples from QuANTUM-First analyzed for *FLT3*-ITD MRD by PCR-NGS, we sought to answer the following questions:
  - Do deeper remissions, defined as having lower *FLT3*-ITD MRD at defined therapy time points, correlate with survival?
  - Does the addition of quizartinib to intensive chemotherapy result in lower levels of *FLT3*-ITD MRD (eg, deeper remissions)?
  - Does ITD length at diagnosis impact the outcome and if so, what is the impact of quizartinib on these outcomes?
  - Does the presence of multiple ITD clones at diagnosis impact the outcome, and if so, what is the impact of quizartinib on these outcomes?

METHODS

MRD Assay

- *FLT3*-ITD mutations were obtained from 800 ng to 1100 ng of genomic DNA isolated from bone marrow aspirates (99.4%) or peripheral blood (8.4%) from patients after achieving remission after 1 or 2 induction courses
- DNA was analyzed using a *FLT3*-ITD PCR-NGS assay specifically developed for this trial (**Figure 3**)[6,15]
  - PCR was used to amplify a region of *FLT3*-ITD between exon 14 and exon 15 in patient samples and then subsequently sequenced after library preparation using the Illumina MiSeq System, where the lower limit of quantification (LLOQ) was 10^{-4} and the estimated lower limit of detection was ~$2{\cdot}10^{-6}$
- Length and sequence of any *FLT3*-ITD mutations detected after induction were cross-validated against the *FLT3*-ITD detected at enrollment for each patient
- Using a custom bioinformatics program, VAF were calculated with sensitivity of $5{\times}10^{-5}$
- The assay can identify multiple ITD sequences
- Two cutoffs for MRD analyses were used:
  - 10^{-4} leukemia cells (predefined/per protocol, based on the assay LLOQ)
  - Zero/undetectable (post hoc analysis)

Figure 3. Schema for *FLT3*-ITD MRD Assay



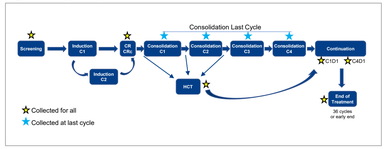
Ultra-deep sequencing of the region (Illumina SBS)

CE, capillary electrophoresis; *FLT3*-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; ITD, internal tandem duplication; MRD, measurable residual disease; NGS, next-generation sequencing; PCR, polymerase chain reaction.

Sample Acquisition/Collection

- We performed MRD analyses at the following 3 time points (**Figure 4**):
  - "End of induction": samples were collected up to end of induction at the time of response assessment, before any further therapy
  - "After 2 cycles of chemotherapy": samples were collected up to end of 2 cycles of chemotherapy, defined as 2 cycles of induction chemotherapy or 1 cycle of induction chemotherapy + 1 cycle of consolidation chemotherapy
  - "After last consolidation cycle": samples were collected up to end of consolidation, before transplant or before continuation C1D1 for transplant patients and before continuation C1D1 for nontransplant patients; if there was no MRD data for the last consolidation cycle, the earlier available MRD status was used, including from induction

Figure 4. Sample Acquisition/Collection



C, cycle; D, day; CR, complete remission; CRc, composite complete remission.

Statistical Methods

- Comparison of the *FLT3*-ITD MRD VAF between treatment arms across time points was made using a Wilcoxon rank-sum test
- Comparisons of OS by ITD length and number of ITD inserts were made using unstratified Cox regression analysis
- All *P* values were not adjusted for multiplicity

RESULTS

Patient Characteristics

- Baseline characteristics of the patients who achieved CRc by the end of induction are very typical for *FLT3*-ITD AML: median age of 55-56 years and broad range of *FLT3* mutation burden at diagnosis (**Table 3**)
- There were 47 patients who achieved CRc by the end of induction without MRD data, and these had similar characteristics as the patients with MRD data

Table 3. Baseline Characteristics of Patients With CRc by the End of Induction

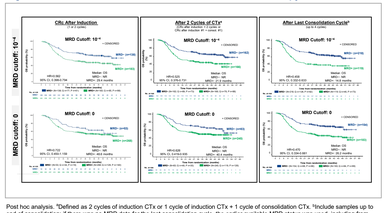
Patient characteristics	Patients who achieved CRc by the end of induction (N=368; 192 with quizartinib, 176 with placebo)	
	With available MRD data (N=321)[a]	
	Quizartinib (N=162)	Placebo (N=159)
Age, years		
Median (range)	56 (23-75)	55 (20-75)
<60 years, n (%)	99 (61.1)	96 (60.4)
≥60 years, n (%)	63 (38.9)	63 (39.6)
60-64 years, n (%)	20 (12.3)	25 (15.7)
≥65 years, n (%)	43 (26.5)	38 (23.9)
Sex, n (%)		
Male	73 (45.1)	65 (40.9)
Female	89 (54.9)	94 (59.1)
ECOG PS, n (%)		
0	53 (32.7)	56 (35.2)
1	83 (51.2)	82 (51.6)
2	26 (16.0)	21 (13.2)
Mutated *NPM1*, n (%)	99 (61.1)	102 (64.2)
Mutated *CEBPA*, n (%)	37 (22.8)	39 (24.5)
***FLT3*-ITD/total FLT3, n (%)**		
≥3% to ≤25%	57 (35.2)	50 (31.4)
>25% to ≤50%	85 (52.5)	88 (55.3)
>50%	20 (12.3)	21 (13.2)
>25%	105 (64.8)	109 (68.6)
Unknown	0	0
MRD sample collection, n (%)		
Peripheral blood	16 (9.9)	11 (6.9)
Bone marrow aspirate	161 (99.4)	158 (99.4)

[a]MRD data are available for 321 out of 368 patients achieving CRc after 1 or 2 courses of induction (47 patients had no MRD data).; *CEBPA*, CCAAT enhancer-binding protein alpha; ECOG PS, ECOG PS, Eastern Cooperative Oncology Group performance status; CRc, composite complete remission; *FLT3*-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; MRD, measurable residual disease; *NPM1*, nucleophosmin 1.

Effect of *FLT3*-ITD MRD on OS

- Analysis of OS in patients who achieved CRc by the end of induction, based on MRD status, using either the 10^{-4} cutoff or the 0 cutoff, across the 3 time points show that a lower level of *FLT3*-ITD MRD confers a survival benefit, regardless of treatment arm (**Figure 5**)

Figure 5. *FLT3*-ITD MRD Reduction Predicts OS Across Therapy Time Points

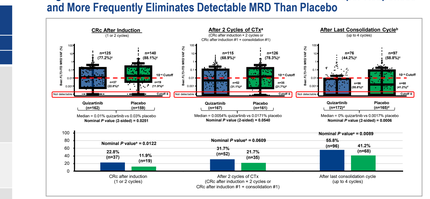


Post hoc analysis. *Defined as 2 cycles of induction CTx or 1 cycle of induction CTx + 1 cycle of consolidation CTx. †Include samples up to end of consolidation; if there was no MRD data for the last consolidation cycle, the earlier available MRD status was used, including from induction. CRc, composite complete remission; CTx, chemotherapy; *FLT3*-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; HR, hazard ratio; MRD, measurable residual disease; NR, not reached; OS, overall survival; P, placebo; Q, quizartinib.

Effect of Quizartinib on *FLT3*-ITD MRD

- Among patients with CRc at end of induction, the median best *FLT3*-ITD VAF by end of induction was 3 times lower with quizartinib versus placebo (**Figure 6**)
- Similar results were seen at later time points: after 2 cycles of chemotherapy and by the end of consolidation
- The percentages of patients with undetectable MRD are consistently higher in the quizartinib arm across therapy time points

Figure 6. Across the Treatment Course, Quizartinib Leads to Deeper Responses and More Frequently Eliminates Detectable MRD Than Placebo

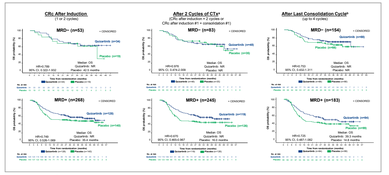


Post hoc analysis. *Defined as 2 cycles of induction CTx or 1 cycle of induction CTx + 1 cycle of consolidation CTx. †Include samples up to end of consolidation, including from induction. ‡Percentage of patients with *FLT3*-ITD MRD VAF>0 among CRc patients with MRD data. §Percentage of patients with *FLT3*-ITD MRD VAF>0 among CRc patients with MRD data. ¶Fisher's exact test. CRc, composite complete remission; CTx, chemotherapy; *FLT3*-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; MRD, measurable residual disease; VAF, variant allele frequency.

Effect of *FLT3*-ITD MRD on OS, by Treatment Arm

- Analysis of OS in patients who achieved CRc by the end of induction, based on MRD status, and by treatment arm, using the 0 cutoff, across the 3 time points show that quizartinib confers a survival benefit regardless of MRD status, but especially among MRD+ patients (**Figure 7**)

Figure 7. *FLT3*-ITD MRD Reduction Predicts Survival Across Therapy Time Points (Cutoff 0)

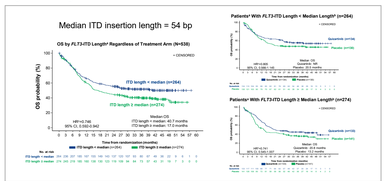


Post hoc analysis. *Defined as 2 cycles of induction CTx or 1 cycle of induction CTx + 1 cycle of consolidation CTx. †Include samples up to end of consolidation; if there was no MRD data for the last consolidation cycle, the earlier available MRD status was used, including from induction. CRc, composite complete remission; CTx, chemotherapy; *FLT3*-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; HR, hazard ratio; MRD, measurable residual disease; NR, not reached; OS, overall survival.

OS by *FLT3*-ITD Length

- The OS analysis by *FLT3*-ITD length regardless of treatment arm shows that having ITD longer than the median length confers a worse survival (**Figure 8**)
- The OS analysis by *FLT3*-ITD length and by treatment arm shows that quizartinib treatment provides a survival benefit over placebo in both patients with long ITDs and short ITDs

Figure 8. Long ITD Insertions Are Associated With Worse Survival

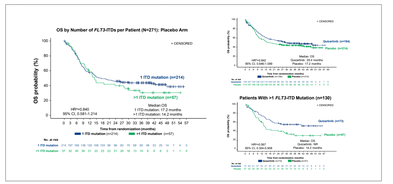


Post hoc analysis. *Patients may have only 1 ITD length or >1 ITD length. †Median ITD length (54 bp) is calculated based on enrollment assay data (Navigate BioPharma *FLT3*-ITD Mutation Assay). ‡Unstratified Cox regression analysis.
FLT3-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; HR, hazard ratio; ITD, internal tandem duplication; NR, not reached; OS, overall survival.

OS by Number of *FLT3*-ITDs

- The OS analysis by number of *FLT3*-ITD inserts in the placebo arm shows that having multiple ITDs confers a worse survival (**Figure 9**)
- The OS analysis by number of *FLT3*-ITD inserts and by treatment arm shows that quizartinib treatment provides a survival benefit over placebo, especially among patients with multiple ITDs

Figure 9. Multiple ITDs Are Associated With Worse Survival, and Quizartinib Can Improve OS in Patients With Multiple ITDs



Post hoc analysis. *Unstratified Cox regression analysis.
FLT3-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; HR, hazard ratio; ITD, internal tandem duplication; NR, not reached; OS, overall survival.

SUMMARY

- These findings demonstrate the potential prognostic utility of *FLT3*-ITD–specific MRD measurements in the clinical management of patients with *FLT3*-ITD+ AML
- The elimination of detectable *FLT3*-ITD MRD is associated with longer OS compared with intensive chemotherapy with or without quizartinib
- Therapy with quizartinib is associated with deeper responses and more frequently eliminates detectable MRD than placebo after induction, following 2 cycles of chemotherapy, and after consolidation
- The presence of multiple ITDs or long ITD inserts at diagnosis did not negatively impact the survival benefits of quizartinib
- Our data suggest that some of the long-term OS benefits conferred by quizartinib derive from an early, deep, and sustained reduction of the *FLT3*-ITD+ leukemia burden

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ACKNOWLEDGMENT

We would like to thank the patients, their families, and caregivers for their participation in the QuANTUM-First study. We would further like to thank the QuANTUM-First steering committee members, the investigators, Donna Hogge, Jack Hsu, the study staff, and independent review committee and data monitoring committee members for their important contributions. This study is sponsored by Daiichi Sankyo, Inc. Medical writing support was provided by Mohamed Abdelmegeed, MD, PhD, CMPP, Emily Cullinan PhD, CMPP, and Francesca Balordi, PhD, CMPP, of The Lockwood Group (Stamford, CT, USA), in accordance with Good Publication Practice (GPP 2022) guidelines, with funding by Daiichi Sankyo, Inc.

Presented at the 28th Annual International Congress on Hematologic Malignancies®: Focus on Leukemias, Lymphomas, and Myeloma (ICHM); February 29, 2024-March 3, 2024; Miami, FL
This poster is an encore of the ASH 2023 oral presentation (Publication Number: 832)

The following authors, all from Daiichi Sankyo, Inc, were not included as authors in this poster, due to ICHM abstract requirements: Jaime E. Connolly Rohrbach, Ken C.N. Chang, Li Liu, Yasser Mostafa Kamel, Karima Imadalou, Abderrahmane Laadem, and Arnaud Lesegretain