QuANTUM-First: Clinical Bridging Study for FMS-Like Tyrosine Kinase 3–Internal Tandem Duplication (*FLT3*-ITD) Companion Diagnostic Development

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BACKGROUND

- The phase 3 study QuANTUM-First (NCT02668653) showed that the highly potent, selective, type 2 FMS-like tyrosine kinase 3 (FLT3) inhibitor quizartinib in combination with standard chemotherapy and/or allogeneic hematopoietic cell transplantation (allo-HCT), followed by quizartinib monotherapy for up to 36 cycles (~3 years), reduced the relative risk of death by 22.4% versus placebo in newly diagnosed *FLT3*-internal tandem duplication (ITD)–positive acute myeloid leukemia (AML; hazard ratio [HR], 0.776; 95% CI, 0.615-0.979; *P*=0.0324)[1]
  - Quizartinib has recently been approved by the United States (US) Food and Drug Administration (FDA),[2,3] the Japanese health agency,[4] and the European Medicines Agency.[5] Quizartinib is approved in combination with chemotherapy across induction, consolidation, and as maintenance monotherapy (but not after allo-HCT in the US) for the treatment of adult patients with newly diagnosed *FLT3*-ITD–positive AML as detected by the LeukoStrat® CDx *FLT3* Mutation Assay (Invivoscribe, Inc., San Diego, CA) as the companion diagnostic (CDx)[6,7]
- The LeukoStrat CDx *FLT3* Mutation Assay is a polymerase chain reaction (PCR)–based, in vitro diagnostic test designed to detect ITD mutations and tyrosine kinase domain mutations D835 and I836 in the *FLT3* gene in genomic DNA extracted from mononuclear cells obtained from peripheral blood or bone marrow aspirates of patients with AML[8]
- The LeukoStrat CDx *FLT3* Mutation Assay is approved for the selection of patients with AML and *FLT3*-ITD mutations for treatment with quizartinib in the first-line setting (in the US, Japan and the EU) and in the relapsed/refractory setting (in Japan), as well as with other FLT3 inhibitors[8]
- In QuANTUM-First, *FLT3*-ITD mutation status was determined using a *FLT3*-ITD mutation detection clinical trial assay (CTA) validated under design control by Navigate BioPharma Services, Inc.[1]
- Here, we present the data of the QuANTUM-First bridging study conducted to demonstrate agreement with respect to selection of patients that are *FLT3*-ITD–positive between the CTA and the LeukoStrat CDx *FLT3* Mutation Assay (ITD portion only)

OBJECTIVES

- Concordance analysis: to assess agreement between the CTA and the LeukoStrat CDx *FLT3* Mutation Assay in *FLT3*-ITD–positive patient selection
- Efficacy analysis: to retrospectively determine whether quizartinib efficacy (overall survival [OS]) was maintained in newly diagnosed *FLT3*-ITD–positive patients with AML from QuANTUM-First, when patients had been selected using the LeukoStrat CDx *FLT3* Mutation Assay compared with CTA

METHODS

Molecular Assay Description

- In both CTA and CDx, DNA extracted from bone marrow (n=884 each) or peripheral blood (n=139 each) was amplified via PCR and the amplicons were detected via capillary electrophoresis
- A sample was considered CTA positive (CTA+) if the variant allele frequency (*FLT3*-ITD/total *FLT3*) was ≥3% and CDx positive (CDx+) if the signal ratio (*FLT3*-ITD/*FLT3* wild type) was ≥0.05

Study Design and Patient Samples

- The agreement between CTA and CDx was based on evaluating CTA+ and CTA-negative (CTA−) samples with the LeukoStrat CDx *FLT3* Mutation Assay
- A primary analysis included the CDx detected (CDx+ and CDx-negative [CDx−]) and the CDx invalid results
- A secondary analysis used CDx+ and CDx− results only
- To establish an agreement between CTA and CDx, positive percent agreement (PPA) and negative percent agreement (NPA) were determined using CTA results as reference for the agreement analysis set (AAS)

Statistical Analyses

- Concordance was established if the lower bounds of the 95% CIs for both PPA and NPA was greater than or equal to 90% for the analysis that included the invalid CDx results
- Prevalence-simulated performance of the CDx versus CTA was used to determine true positive and true negatives as follows:
  - Positive predictive value (PPV) Pr(CTA+/CDx+)
  - Negative predictive value (NPV) Pr(CTA−/CDx−)
- Median OS in the subgroups was calculated based on Kaplan-Meier analysis
- Stratified Cox proportional hazards regression model was used to estimate HRs, 95% CI, and *P* value

RESULTS

Patient Samples

- The full analysis set (N=3468) included all QuANTUM-First screened CTA+ patients (n=863), all screened CTA− patients (n=2556), and patients with unknown CTA status not eligible for randomization due to other criteria (n=49; **Table 1**)
  - Of these, 1032 patients formed the primary analysis set (PAS), including all patients randomized in QuANTUM-First with samples available for CDx testing (N=513; quizartinib, n=254; placebo, n=259) and a randomly selected subset of CTA− patients (n=519)
  - The ascertainment rate was 95.2% (513/539), as 26 of the 539 patients randomized in QuANTUM-First were excluded from the bridging study

Table 1. Accountability for All Screened Patients in the QuANTUM-First Trial (Full Analysis Set)

Characteristics	Total patients, n	Samples sent to be tested by CDx, n
QuANTUM-First patients screened for eligibility	3468	1032
Tested as CTA+	863	513[a]
Randomized in quizartinib arm	267	254
Treated in quizartinib arm	264	251
Randomized in placebo arm	271	259
Treated in placebo arm	268	256
Not randomized	325	0
Not selected for bridging study	350	0
Tested as CTA−	2556	519[b]
Selected in bridging study	519[b]	519[b]
Not selected for bridging study	2037	0
Not tested by CTA	49	0
Not tested but randomized	1	0
CTA result invalid	0	0

[a]Twenty-six of the 539 patients randomized in QuANTUM-First were excluded from the bridging study owing to sample availability, informed consent form restrictions, and sample destruction requests.
[b]A total of 519 CTA− samples were sent to be tested by CDx, but 3 of those did not proceed with testing because of insufficient volume/DNA amount.
CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; CTA−, clinical trial assay negative; CTA+, clinical trial assay positive.

- The agreement analysis set (AAS, [N=1029; CTA+, n=513; CTA−, n=516]) included patients in the primary analysis set with valid CTA results and tested with CDx. A summary of baseline demographics and disease characteristics of the CDx-analyzed AAS patients are shown in **Table 2**
  - Six samples (3 CTA+, 3 CTA−) did not yield valid CDx results, resulting in 1023 CDx-evaluable total patients (CTA+, n=510; CTA−, n=513)

Table 2. Baseline Demographics and Disease Characteristics of the CDx-Analyzed Population (Agreement Analysis Set)

Characteristics	CTA+				CTA−			
	CDx+ (n=483)	CDx− (n=27)	CDx invalid (n=3)	Total (N=513)	CDx+ (n=0)	CDx− (n=513)	CDx invalid (n=3)	Total (N=516)[b]
Age[a]								
Median (range), years	56 (20-75)	57 (20-72)	49 (48-72)	56 (20-75)	0	57 (18-75)	72 (48-74)	57 (18-75)
<60 years, n (%)	284 (58.8)	15 (55.6)	2 (66.7)	301 (58.7)	0	133 (25.9)	0	133 (25.8)
≥60 years, n (%)	199 (41.2)	12 (44.4)	1 (33.3)	212 (41.3)	0	93 (18.1)	0	93 (18.0)
Missing, n (%)	0	0	0	0	0	287 (55.9)	3 (100)	290 (56.2)
Sex, n (%)								
Male	218 (45.1)	12 (44.4)	2 (66.7)	232 (45.2)	0	230 (44.8)	1 (33.3)	231 (44.8)
Female	265 (54.9)	15 (55.6)	1 (33.3)	281 (54.8)	0	283 (55.2)	2 (66.7)	285 (55.2)
Race, n (%)								
White	299 (61.9)	19 (70.4)	1 (33.3)	319 (62.2)	0	318 (62.0)	2 (66.7)	320 (62.0)
Black or African American	6 (1.2)	1 (3.7)	0	7 (1.4)	0	7 (1.4)	0	7 (1.4)
Asian	127 (26.3)	6 (22.2)	2 (66.7)	135 (26.3)	0	137 (26.7)	0	137 (26.6)
American Indian or Alaska Native	1 (0.2)	0	0	1 (0.2)	0	0	0	0
Native Hawaiian or other Pacific Islander	0	0	0	0	0	0	1 (33.3)	1 (0.2)
Other	50 (10.4)	1 (3.7)	0	51 (9.9)	0	51 (9.9)	0	51 (9.9)
Ethnicity, n (%)								
Hispanic or Latino	20 (4.1)	2 (7.4)	0	22 (4.3)	0	34 (6.6)	1 (33.3)	35 (6.8)
Not Hispanic or Latino	418 (86.5)	24 (88.9)	3 (100)	445 (86.7)	0	440 (85.8)	2 (66.7)	442 (85.7)
Not collected per local regulations	45 (9.3)	1 (3.7)	0	46 (9.0)	0	33 (6.4)	0	33 (6.4)
Region, n (%)								
North America	33 (6.8)	1 (3.7)	0	34 (6.6)	0	36 (7.0)	0	36 (7.0)
Europe	305 (63.1)	17 (63.0)	1 (33.3)	323 (63.0)	0	322 (62.8)	2 (66.7)	324 (62.8)
Asia/other regions	145 (30.0)	9 (33.3)	2 (66.7)	156 (30.4)	0	155 (30.2)	1 (33.3)	156 (30.2)
ECOG PS score, n (%)								
0	163 (33.7)	13 (48.1)	2 (66.7)	178 (34.7)	N/A	N/A	N/A	N/A
1	241 (49.9)	12 (44.4)	0	253 (49.3)	N/A	N/A	N/A	N/A
2	78 (16.1)	2 (7.4)	1 (33.3)	81 (15.8)	N/A	N/A	N/A	N/A
Missing	1 (0.2)	0	0	1 (0.2)	N/A	N/A	N/A	N/A

[a]Age in years is calculated using the birth date as of informed consent date.
[b]A total of 519 CTA− samples were sent to be tested by CDx, but 3 of those did not proceed with testing due to quantity being insufficient.
CDx, LeukoStrat CDx *FLT3* Mutation Assay; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; CDx−, companion diagnostic negative; CDx+, companion diagnostic positive; CTA−, clinical trial assay negative; CTA+, clinical trial assay positive; ECOG PS, Eastern Cooperative Oncology Group performance status; N/A, not available.

Concordance Analysis

- Among 510 CTA+ samples, 483 were CDx+. Among 513 CTA− samples, 513 were CDx−. Therefore, 996 samples yielded concordant results, 27 samples yielded discordant results, and 6 samples did not yield a valid CDx result for assay comparison (**Table 3**)

Table 3. Contingency Between CTA and CDx (Agreement Analysis Set)

CDx	CTA	
	CTA+, n	CTA−, n
CDx+	483	0
CDx−	27	513
Invalid[a]	3	3
Total	513	516

[a]Invalid means that a sample was tested on the CDx assay but failed to return a valid result.
CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CDx−, companion diagnostic negative; CDx+, companion diagnostic positive; CTA−, clinical trial assay negative; CTA+, clinical trial assay positive; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay.

- Point estimates of PPA and NPA were 94.2% and 99.4%, respectively, with invalid CDx results included in the calculation, and 94.7% and 100%, respectively, without invalid CDx results. The lower bounds of the 95% CIs were all above the corresponding acceptance criterion of 90% for PPA and NPA (**Table 4**)
- In QuANTUM-First, the prevalence of CTA+ was 24.9% among screened patients (863/3468), whereas in the bridging study, 49.9% (510/1023) of patients were CTA+; this enrichment in CTA+ patients could lead to a biased estimate of the agreement between CTA and CDx when using CDx as reference
- The PPV and NPV of the CDx adjusted for this enrichment ± invalid CDx results showed that the lower bounds of the 95% CIs were all above 95% (**Table 4**)

Table 4. Agreement and Predictive Values Between CTA and CDx

Measure of agreement based on CTA results (Agreement Analysis Set)	Without invalid CDx results		With invalid CDx results[a]	
	Percent agreement (n/N)	95% CI[b]	Percent agreement (n/N)	95% CI[b]
PPA	94.7 (483/510)	92.4-96.5	94.2 (483/513)	91.8-96.0
NPA	100 (513/513)	99.3-100	99.4 (513/516)	98.3-99.9
OPA	97.4 (996/1023)	96.2-98.3	96.8 (996/1029)	95.5-97.8
Predictive values based on CDx results adjusted for enrichment[c]	**Without invalid CDx results**		**With invalid CDx results[a]**	
	Predictive values, %	95% CI[d]	Predictive values, %	95% CI[d]
PPV	100	100-100	98.2	95.9-100
NPV	98.3	97.6-98.9	98.1	97.4-98.7

[a]Invalid CDx results used as discordant results.
[b]Calculated using the exact (Clopper-Pearson) method.
[c]Prevalence of CTA+ set as 25% (to account for bridging bias).
[d]Calculated using nonparametric bootstrapping method.
CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; CTA+, clinical trial assay positive; PPA, positive percent agreement; PPV, positive predictive value; NPA, negative percent agreement; NPV, negative predictive value; OPA, overall percent agreement.

Clinical Efficacy Analysis

- Baseline demographics and disease characteristics were balanced between the 2 treatment arms in the intent-to-treat (ITT) CDx+ population (ITT CDx+=CTA+ CDx+ [N=483]; quizartinib, n=242; placebo, n=241; **Table 5**)

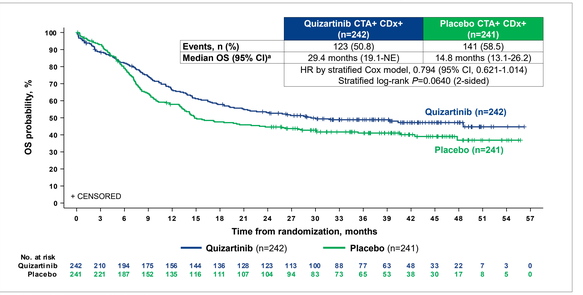
Table 5. Baseline Demographics and Disease Characteristics of the Intent-to-Treat CDx+ Analysis Set (CTA+ CDx+)

Characteristics	Quizartinib (n=242)	Placebo (n=241)	All (N=483)
Age[a]			
Median (range), years	56 (23-75)	57 (20-75)	56 (20-75)
<60 years, n (%)	145 (59.9)	139 (57.7)	284 (58.8)
≥60 years, n (%)	97 (40.1)	102 (42.3)	199 (41.2)
Sex, n (%)			
Male	113 (46.7)	105 (43.6)	218 (45.1)
Female	129 (53.3)	136 (56.4)	265 (54.9)
Race, n (%)			
White	151 (62.4)	148 (61.4)	299 (61.9)
Black or African American	2 (0.8)	4 (1.7)	6 (1.2)
Asian	62 (25.6)	65 (27.0)	127 (26.3)
American Indian or Alaska Native	0	1 (0.4)	1 (0.2)
Native Hawaiian or other Pacific Islander	0	0	0
Other	27 (11.2)	23 (9.5)	50 (10.4)
Ethnicity, n (%)			
Hispanic or Latino	7 (2.9)	13 (5.4)	20 (4.1)
Not Hispanic or Latino	212 (87.6)	206 (85.5)	418 (86.5)
Not collected per local regulations	23 (9.5)	22 (9.1)	45 (9.3)
Region, n (%)			
North America	16 (6.6)	17 (7.1)	33 (6.8)
Europe	156 (64.5)	149 (61.8)	305 (63.1)
Asia/other regions	70 (28.9)	75 (31.1)	145 (30.0)
ECOG PS score, n (%)			
0	78 (32.2)	85 (35.3)	163 (33.7)
1	121 (50.0)	120 (49.8)	241 (49.9)
2	43 (17.8)	35 (14.5)	78 (16.1)
Missing	0	1 (0.4)	1 (0.2)
Cytogenetic risks, n (%)			
Favorable	10 (4.1)	15 (6.2)	25 (5.2)
Intermediate	183 (75.6)	176 (73.0)	359 (74.3)
Unfavorable	19 (7.9)	22 (9.1)	41 (8.5)
Unknown	30 (12.4)	28 (11.6)	58 (12.0)
Mutated *NPM1*,[b] n (%)	134 (55.4)	133 (55.2)	267 (55.3)
Mutated *CEBPA*,[b] n (%)	53 (21.9)	60 (24.9)	113 (23.4)
VAF,[c] n (%)			
≥3% to ≤25%	78 (32.2)	80 (33.2)	158 (32.7)
>25% to ≤50%	135 (55.8)	131 (54.4)	266 (55.1)
>50%	29 (12.0)	30 (12.4)	59 (12.2)

The baseline value is defined as the last nonmissing value before initial administration of study treatment. If >1 race is reported for a patient, he/she is described only in this category and is not described in the other race categories.
[a]Age in years is calculated using the birth date as of informed consent date.
[b]*NPM1* and *CEBPA* data are based on the Navigate BioPharma central data.
[c]VAF was assessed by central laboratory testing.
CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CDx+, companion diagnostic positive; CTA+, clinical trial assay positive; ECOG PS, Eastern Cooperative Oncology Group performance status; VAF, variant allele frequency.

- The efficacy OS analysis in the intent-to-treat CDx+ population demonstrated a clinically relevant OS improvement with quizartinib (median OS of 29.4 months) versus placebo (median OS of 14.8 months), resulting in 14.6 months prolongation of median OS, with an HR of 0.794 (95% CI, 0.621-1.014), corresponding to a 20.6% reduction in relative risk of death (**Figure 1**)

Figure 1. Kaplan-Meier Plot of OS in the Intent-to-Treat CDx+ Analysis Set (CTA+ CDx+)



[a]Calculated using the Brookmeyer and Crowley method.
CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CDx+, companion diagnostic positive; CTA+, clinical trial assay positive; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; HR, hazard ratio; NE, not estimable; OS, overall survival.

ITD Insert Sizes

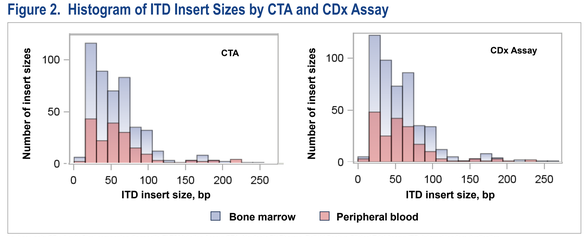
- ITD insert sizes were measured side-by-side in 510 CTA+ samples and in 483 CDx+ samples (**Table 6**, **Figure 2**)
- The ITD insert sizes ranged from 6 base pairs (bp) to 243 bp with the CTA, and from 4 bp to 259 bp with the CDx assay (**Table 6**)
- Most samples with ITD mutations contained insert sizes <100 bp for both the CTA and CDx
- There were 109 CTA and 148 CDx samples containing >1 ITD insert size
- There were 55 CTA and 55 CDx ITD samples that contained insert sizes ≥100 bp, with 33 out of the 55 CTA samples and 26 out of the 55 CDx samples only having insert sizes ≥100 bp (**Table 6**)

Table 6. ITD Insert Sizes Descriptive Statistics by CTA and CDx Assay

Samples with detected ITD mutation, n	CTA (N=510)	CDx (N=483)
Samples with 1 insert size, n	401	335
Samples with >1 insert size, n	109	148
Minimum insert size, bp	6	4
Mean insert size, bp	56.33	55.96
Maximum insert size, bp	243	259
Samples with ITD <100 bp only, n	455	428
Samples with ITD ≥100 bp only, n	33	26

bp, base pair; CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; ITD, internal tandem duplication.

Figure 2. Histogram of ITD Insert Sizes by CTA and CDx Assay



bp, base pair; CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; ITD, internal tandem duplication.

CONCLUSIONS

- Acceptance criteria were met demonstrating agreement between the Navigate BioPharma Daiichi Sankyo CTA and the LeukoStrat CDx *FLT3* Mutation Assay in identifying newly diagnosed *FLT3*-ITD–positive patients with AML
- OS benefit provided by quizartinib in the intent-to-treat CDx+ (CTA+ CDx+) population is comparable with the OS benefit in the intent-to-treat population of QuANTUM-First
- Data from this bridging study led to the recent approval by the FDA and the label expansion in Japan of the LeukoStrat CDx *FLT3* Mutation Assay for in vitro diagnostic use in the selection of AML patients with *FLT3*-ITD mutations for treatment with quizartinib in the first-line setting[6-8]

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QuANTUM-FIRST STUDY

Background, Objectives, and Methods

BACKGROUND

- Based on the QuANTUM-First study (NCT02668653) data[1]:
  - Quizartinib has been approved in the US,[2,3] in Japan[4] and in the EU[5] in combination with chemotherapy across induction, consolidation, and as maintenance monotherapy (but not after transplantation in the US) for the treatment of adult patients with newly diagnosed *FLT3*-ITD–positive AML as detected by the LeukoStrat® CDx *FLT3* Mutation Assay (Invivoscribe, Inc., San Diego, CA) as the companion diagnostic (CDx)[3-7]
- The LeukoStrat CDx *FLT3* Mutation Assay is approved for the selection of patients with AML and *FLT3*-ITD mutations for treatment with quizartinib in the first-line setting (in the US, Japan and the EU) and in relapsed/refractory (in Japan), as well as with other FLT3 inhibitors[8]
- In QuANTUM-First, *FLT3*-ITD mutation status was determined using a *FLT3*-ITD mutation detection clinical trial assay (CTA) validated under design control by Navigate BioPharma Services, Inc.[1]

OBJECTIVES

- The objectives of this bridging study were to assess agreement between the CTA and the CDx in *FLT3*-ITD–positive patient selection and to retrospectively determine if quizartinib efficacy (OS) was maintained in newly diagnosed *FLT3*-ITD–positive patients with AML from QuANTUM-First, when patients had been selected using the CDx compared with CTA

METHODS

- In both CTA and CDx, DNA extracted from bone marrow (n=884 each) or peripheral blood (n=139 each) was amplified via polymerase chain reaction and the amplicons were detected via capillary electrophoresis
- A sample was considered CTA positive (CTA+) if the variant allele frequency (*FLT3*-ITD/total *FLT3*) was ≥3%
- A sample was considered CDx positive (CDx+) if the signal ratio (*FLT3*-ITD/*FLT3* wild type) was ≥0.05

AML, acute myeloid leukemia; EU, European Union; *FLT3*-ITD, FMS-like tyrosine kinase 3–internal tandem duplication; US, United States; OS, overall survival.

1. Erba HP, et al. *Lancet.* 2023;401(10388):1571-1583. **2.** Daiichi Sankyo press release. VANFLYTA® first FLT3 inhibitor approved in the U.S. specifically for patients with newly diagnosed *FLT3*-ITD positive AML. Accessed July 31, 2023. https://www.daiichisankyo.com/files/news/pressrelease/pdf/202307/20230720_E.pdf. **3.** VANFLYTA® (quizartinib) package insert. Daiichi Sankyo, Inc. July 2023. **4.** Daiichi Sankyo press release. VANFLYTA® first FLT3 inhibitor approved in Japan for patients with newly diagnosed *FLT3*-ITD positive AML. Accessed June 13, 2023. https://www.daiichisankyo.com/files/news/pressrelease/pdf/202305/20230525_E.pdf. **5.** Daiichi Sankyo press release. VANFLYTA® approved in the EU as the first FLT3 inhibitor specifically for patients with newly diagnosed FLT3-ITD positive AML. Accessed November 9, 2023. https://www.daiichisankyo.com/files/news/pressrelease/pdf/202311/20231109_E.pdf. **6.** Invivoscribe, Inc. Invivoscribe announces FDA approval of the LeukoStrat® CDx *FLT3* Mutation Assay to select patients with *FLT3*-ITD positive AML for treatment with VANFLYTA®. Accessed October 12, 2023. https://invivoscribe.com/invivoscribe-announces-fda-approval-of-the-leukostrat-cdx-flt3-mutation-assay-to-select-patients-with-flt3-itd-positive-aml-for-treatment-with-vanflyta/. **7.** Invivoscribe, Inc. Invivoscribe announces updated reimbursement for the LeukoStrat CDx *FLT3* Mutation Assay® to select newly diagnosed *FLT3*-ITD positive AML patients eligible for VANFLYTA® in Japan. Accessed October 12, 2023. https://invivoscribe.com/invivoscribe-announces-updated-reimbursement-for-the-leukostrat-cdx-flt3-mutation-assay-to-select-newly-diagnosed-flt3-itd-positive-aml-patients-eligible-for-vanflyta-in-japan/. **8.** The LeukoStrat® CDx *FLT3* Mutation Assay. Accessed October 12, 2023. https://invivoscribe.com/products/companion-diagnostics-cdx/.

Results: Patient Samples

Accountability for All Screened Patients in the QuANTUM-First Trial (FAS)

Characteristics	Total patients, n	Samples sent to be tested by CDx, n
QuANTUM-First patients screened for eligibility	3468	1032
Tested as CTA+	863	513[a]
Randomized in quizartinib arm	267	254
Treated in quizartinib arm	264	251
Randomized in placebo arm	271	259
Treated in placebo arm	268	256
Not randomized	325	0
Not selected for bridging study	350	0
Tested as CTA−	2556	519[b]
Selected in bridging study	519[b]	519[b]
Not selected for bridging study	2037	0
Not tested by CTA	49	0
Not tested but randomized	1	0
CTA result invalid	0	0

[a]Twenty-six of the 539 patients randomized in QuANTUM-First were excluded from the bridging study owing to sample availability, informed consent form restrictions, and sample destruction requests.
[b]A total of 519 CTA− samples were sent to be tested by CDx, but 3 of those did not proceed with testing because of insufficient volume/DNA amount.

- Primary analysis set (PAS; N=1032) was defined as patients randomized in QuANTUM-First with samples available for CDx testing (n=513) and a randomly selected subset of CTA− patients (n=519)
- A total of 539 patients enrolled and randomized into QuANTUM-First
  - Twenty-six patients were excluded
  - Ascertainment rate: 95.2% (513/539) at start of the bridging study

Baseline Demographics and Disease Characteristics of the CDx-Analyzed Population (AAS)

Characteristics	CTA+				CTA−			
	CDx+ (n=483)	CDx− (n=27)	CDx invalid (n=3)	Total (N=513)	CDx+ (n=0)	CDx− (n=513)	CDx invalid (n=3)	Total (N=516)[b]
Age[a]								
Median (range), years	56 (20-75)	57 (20-72)	49 (48-72)	56 (20-75)	0	57 (18-75)	72 (48-74)	57 (18-75)
<60 years, n (%)	284 (58.8)	15 (55.6)	2 (66.7)	301 (58.7)	0	133 (25.9)	0	133 (25.8)
≥60 years, n (%)	199 (41.2)	12 (44.4)	1 (33.3)	212 (41.3)	0	93 (18.1)	0	93 (18.0)
Missing, n (%)	0	0	0	0	0	287 (55.9)	3 (100)	290 (56.2)
Sex, n (%)								
Male	218 (45.1)	12 (44.4)	2 (66.7)	232 (45.2)	0	230 (44.8)	1 (33.3)	231 (44.8)
Female	265 (54.9)	15 (55.6)	1 (33.3)	281 (54.8)	0	283 (55.2)	2 (66.7)	285 (55.2)
Region, n (%)								
North America	33 (6.8)	1 (3.7)	0	34 (6.6)	0	36 (7.0)	0	36 (7.0)
Europe	305 (63.1)	17 (63.0)	1 (33.3)	323 (63.0)	0	322 (62.8)	2 (66.7)	324 (62.8)
Asia/other regions	145 (30.0)	9 (33.3)	2 (66.7)	156 (30.4)	0	155 (30.2)	1 (33.3)	156 (30.2)
ECOG PS score, n (%)								
0	163 (33.7)	13 (48.1)	2 (66.7)	178 (34.7)	N/A	N/A	N/A	N/A
1	241 (49.9)	12 (44.4)	0	253 (49.3)	N/A	N/A	N/A	N/A
2	78 (16.1)	2 (7.4)	1 (33.3)	81 (15.8)	N/A	N/A	N/A	N/A
Missing	1 (0.2)	0	0	1 (0.2)	N/A	N/A	N/A	N/A

[a]Age in years is calculated using the birth date as of informed consent date.
[b]A total of 519 CTA− samples were sent to be tested by CDx, but 3 of those did not proceed with testing due to quantity being insufficient.

- AAS (n=1029) was defined as patients in the PAS with valid CTA results and tested with CDx
- Six samples were excluded because of invalid CDx results
  - The total number of CDx-evaluable patients was 1023:
    - CTA+: n=510
    - CTA−: n=513

AAS, agreement analysis set; CDx, LeukoStrat CDx *FLT3* Mutation Assay; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; CDx−, companion diagnostic negative; CDx+, companion diagnostic positive; CTA−, clinical trial assay negative; CTA+, clinical trial assay positive; ECOG PS, Eastern Cooperative Oncology Group performance status; FAS, full analysis set; N/A, not available.

Results: Concordance Analysis

Contingency Between CTA and CDx (AAS)

CDx	CTA	
	CTA+, n	CTA−, n
CDx+	483	0
CDx−	27	513
Invalid[a]	3	3
Total	513	516

[a]Invalid means that a sample was tested on the CDx assay but failed to return a valid result.

- A total of 996 samples yielded concordant results
- A total of 27 samples yielded discordant results
- Six samples did not yield a valid CDx result for assay comparison

Agreement and Predictive Values Between CTA and CDx

Measure of agreement based on CTA results (AAS)	Without invalid CDx results		With invalid CDx results[a]	
	Percent agreement (n/N)	95% CI[b]	Percent agreement (n/N)	95% CI[b]
PPA	94.7 (483/510)	92.4-96.5	94.2 (483/513)	91.8-96.0
NPA	100 (513/513)	99.3-100	99.4 (513/516)	98.3-99.9
OPA	97.4 (996/1023)	96.2-98.3	96.8 (996/1029)	95.5-97.8
Predictive values based on CDx results adjusted for enrichment[c]	**Without invalid CDx results**		**With invalid CDx results[a]**	
	Predictive values, %	**95% CI[d]**	**Predictive values, %**	**95% CI[d]**
PPV	100	100-100	98.2	95.9-100
NPV	98.3	97.6-98.9	98.1	97.4-98.7

[a]Invalid CDx results used as discordant results.
[b]Calculated using the exact (Clopper-Pearson) method.
[c]Prevalence of CTA+ set as 25% (to account for bridging bias).
[d]Calculated using nonparametric bootstrapping method.

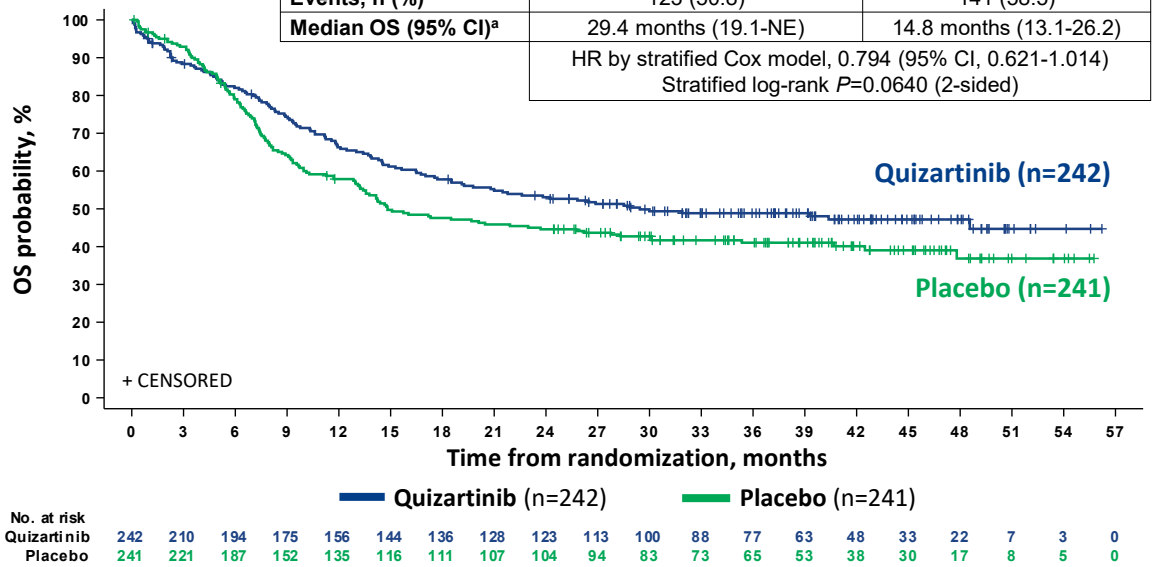
- LeukoStrat CDx *FLT3* Mutation Assay met the prespecified requirements for PPA and NPA for detection of *FLT3*-ITD:
  - 94.7% PPA without invalids
  - 100% NPA without invalids
  - The lower bounds of the 95% CIs for PPA and NPA were above the acceptance criterion of 90%
- In QuANTUM-First, the prevalence of CTA+ was 24.9% among screened patients (863/3468), whereas in the bridging study, 49.9% (510/1023) of patients were CTA+: this enrichment in CTA+ patients could lead to a biased estimate of the agreement between CTA and CDx when using CDx as reference
- The PPV and NPV of the CDx adjusted for this enrichment ± invalid CDx results showed that the lower bounds of the 95% CIs were all above 95%

AAS, agreement analysis set; CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; CTA+, clinical trial assay positive; PPA, positive percent agreement; PPV, positive predictive value; NPA, negative percent agreement; NPV, negative predictive value; OPA, overall percent agreement.

Results: Clinical Efficacy Analysis

Kaplan-Meier Plot of OS in the ITT CDx+ Analysis Set (CTA+ CDx+)

	Quizartinib CTA+ CDx+ (n=242)	Placebo CTA+ CDx+ (n=241)
Events, n (%)	123 (50.8)	141 (58.5)
Median OS (95% CI)[a]	29.4 months (19.1-NE)	14.8 months (13.1-26.2)
	HR by stratified Cox model, 0.794 (95% CI, 0.621-1.014) Stratified log-rank *P*=0.0640 (2-sided)	



[a]Calculated using the Brookmeyer and Crowley method.

- In the ITT CDx+ population, there was a clinically relevant OS improvement with quizartinib versus placebo, comparable with the OS benefit in the ITT population of QuANTUM-First
  - HR of 0.794 (*P*=0.0640), similar to the OS primary analysis (HR, 0.776; *P*=0.0324)
  - 20.6% relative risk reduction of death in favor of quizartinib, similar to 22.4% of the OS primary analysis

ITD Insert Sizes Descriptive Statistics by CTA and CDx Assay

Samples with detected ITD mutation, n	CTA (N=510)	CDx (N=483)
Samples with 1 insert size, n	401	335
Samples with >1 insert size, n	109	148
Minimum insert size, bp	6	4
Mean insert size, bp	56.33	55.96
Maximum insert size, bp	243	259
Samples with ITD <100 bp only, n	455	428
Samples with ITD ≥100 bp only, n	33	26

- Most samples with ITD mutations contained insert sizes <100 bp for both assays
- There were 109 CTA and 148 CDx samples containing >1 ITD insert size
- There were 55 CTA and 55 CDx ITD samples contained insert sizes ≥100 bp, with 33/55 CTA samples and 26/55 CDx samples only having insert sizes ≥100 bp

CDx, LeukoStrat companion diagnostic *FLT3* Mutation Assay; CDx+, companion diagnostic positive; CTA+, clinical trial assay positive; CTA, Navigate BioPharma, Daiichi Sankyo Clinical Trial Assay; HR, hazard ratio; ITD, internal tandem duplication; ITT, intent-to-treat; NE, not estimable; OS, overall survival.

Conclusions

- Acceptance criteria were met demonstrating agreement between the Navigate BioPharma Daiichi Sankyo CTA and the LeukoStrat CDx *FLT3* Mutation Assay in identifying newly diagnosed *FLT3*-ITD–positive patients with AML
- OS benefit provided by quizartinib in the ITT CDx+ (CTA+ CDx+) population is comparable with the OS benefit in the ITT population of QuANTUM-First
- Data from this bridging study led to the recent approval by the FDA and the label expansion in Japan of the LeukoStrat CDx *FLT3* Mutation Assay for in vitro diagnostic use in the selection of AML patients with *FLT3*-ITD mutations for treatment with quizartinib in the first-line setting[1-3]

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AML, acute myeloid leukemia; CDx, companion diagnostic; CDx+, companion diagnostic positive; CTA, clinical trial assay; CTA+, clinical trial assay positive; FDA, United States Food and Drug Administration; *FLT3*-ITD, FMS-like 3–internal tandem duplication; ITT, intent-to-treat; OS, overall survival.

1. Invivoscribe, Inc. Invivoscribe announces FDA approval of the LeukoStrat CDx *FLT3* mutation assay to select patients with *FLT3*-ITD positive AML for treatment with VANFLYTA®. Accessed October 12, 2023. https://invivoscribe.com/invivoscribe-announces-fda-approval-of-the-leukostrat-cdx-flt3-mutation-assay-to-select-patients-with-flt3-itd-positive-aml-for-treatment-with-vanflyta/. **2.** Invivoscribe, Inc. Invivoscribe announces updated reimbursement for the LeukoStrat CDx *FLT3* mutation assay® to select newly diagnosed *FLT3*-ITD positive AML patients eligible for VANFLYTA® in Japan. Accessed October 12, 2023. https://invivoscribe.com/invivoscribe-announces-updated-reimbursement-for-the-leukostrat-cdx-flt3-mutation-assay-to-select-newly-diagnosed-flt3-itd-positive-aml-patients-eligible-for-vanflyta-in-japan/. **3.** The LeukoStrat® CDx *FLT3* Mutation Assay. Accessed October 12, 2023. https://invivoscribe.com/products/companion-diagnostics-cdx/.