Poster 1424

Concordance Between Bone Marrow and Peripheral Blood Samples for Assessment of *FLT3* Internal Tandem Duplication (ITD) Mutations: Data From Patients Screened for Participation in QuANTUM-R, a Global, Randomized, Open-Label, Phase 3 Study Examining the Effect of Quizartinib Monotherapy vs Salvage Chemotherapy on Overall Survival in Patients With *FLT3*-ITD–Mutated AML Who Are Refractory to or Have Relapsed After First-Line Therapy

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INTRODUCTION

- Acute myeloid leukemia (AML) is a heterogeneous disease characterized by clonal evolution with multiple factors influencing long-term outcome[1,2]
- *FLT3* (FMS-like tyrosine kinase 3) mutations (predominantly internal tandem duplication [ITD]) are reported in ~25% of patients with AML, and *FLT3*-ITD–mutated AML has been associated with poorer outcomes compared with *FLT3* non-mutated AML[3]
- Targeting these mutations with tyrosine kinase inhibitors has become an active area of research
  - The kinase inhibitor, midostaurin, targets a number of kinases, including FLT3 and was recently approved for treatment of newly-diagnosed *FLT3*-mutated AML[4]
  - Several next-generation, more selective FLT3 inhibitors are in late stage clinical development for *FLT3*-mutated AML
    - Quizartinib is an orally administered, next-generation, potent and selective FLT3 inhibitor with promising clinical activity in phase 1-2 trials in relapsed/refractory and newly diagnosed *FLT3*-ITD–mutated AML[5-9]
- Patients must undergo molecular testing to identify the presence of *FLT3* mutations
  - The specimen of choice for such testing is bone marrow (BM; aspirate or biopsy), as the site of origin of the disease
  - However, BM testing is invasive and sometimes cannot be performed repeatedly in a timely manner
- A simple technique using easily obtainable biological specimens from a source such as peripheral blood (PB) may be desirable if the results accurately reflect status in the active disease site (ie, BM)

OBJECTIVE

- Using patient samples from QuANTUM-R (NCT02039726; **Figure 1**), a phase 3 trial investigating the efficacy of quizartinib monotherapy versus salvage chemotherapy in patients with relapsed or refractory *FLT3*-ITD–mutated AML, we investigated whether a PB specimen could be utilized for *FLT3*-ITD determination and if the characteristics and allelic ratio of the ITD mutations identified in PB were consistent with those in BM samples from the same patient

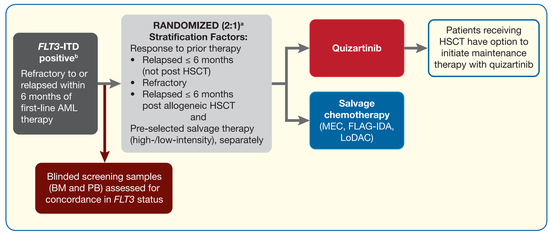

Figure 1. QuANTUM-R study design. Patients receiving MEC or FLAG-IDA will receive 1 cycle of therapy and may receive a second cycle of the same therapy at the Investigator's discretion. Treatment should be discontinued if there is no evidence of response (NR) or progressive disease (PD). For patients who achieve a reduction in blast count but no CR, CRp, or CRi, the investigator should determine whether a second cycle is likely to be beneficial, based on the level of response to the first cycle, toxicity, and performance status. Patients in the quizartinib arm who proceed to HSCT may resume quizartinib 30 to 100 days after HSCT.

Abbreviations: BM, bone marrow; FLAG-IDA, fludarabine, cytarabine, and granulocyte colony-stimulating factor with idarubicin; HSCT, hematopoietic stem cell transplant; LoDAC, low-dose cytarabine; MEC, mitoxantrone, etoposide, and intermediate-dose cytarabine; PB, peripheral blood.

[a]2:1 quizartinib versus salvage chemotherapy (estimated enrollment, n = 363).

[b]Patients with an allelic ratio of *FLT3*-ITD to total *FLT3* of greater than or equal to 3% were included.

METHODS

Patient Samples

- Paired samples of BM and PB were collected from individual patients as part of the screening process for QuANTUM-R
- 1 to 3 mL each of BM and PB were collected on the same day (window Day –14 to Day 0) from each patient prior to receipt of any study treatment
- A statistically powered number of patients (minimum 95) with paired BM and PB samples were selected during a defined period of screening in the phase 3 study

FLT3 Mutation Assay

- Genomic DNA was isolated from EDTA-anticoagulated PB or BM
- PCR primers targeting exons 14 and 15 of the *FLT3* gene were conjugated with fluorescent dyes
- PCR products were separated and detected by capillary electrophoresis. Fragment size analysis was used to resolve and detect different-sized PCR products
  - The non-mutant peak was identified as a single amplification product ~330 bp in size
  - *FLT3*-ITD mutant peaks were identified as amplification products with sizes ≥ 333 bp
  - In each assay run, 4 controls were included: no template control (water), negative control (non-mutant cell line DNA), low positive control, and 100% positive control
- Allelic ratio, for each ITD, was calculated as:

$$\%\ \text{ITD} = \frac{\text{Area under each mutant ITD peak}}{\text{Areas under all mutant ITD peaks + Area under non-mutant peak}}$$

- The assay was validated down to a limit of detection (LoD) of 1%

Statistical Analysis

- Deming Regression was used to find the line of best fit for a 2-dimensional dataset by accounting for potential errors in observations on both sets of values. This is different from simple linear regression where there is one independent and one dependent variable and predicts the dependent variable values as a function of the independent variables
- Bland-Altman difference plot was used to analyze the agreement between samples from the same patient using 2 different assays. This method assumes that if 2 methods are designed to measure the same parameter, in this case the presence of ITD mutations, when samples are chosen with considerable variation in the parameter being measured, the 2 assays should be correlated well

RESULTS

Patient Demographics

- Paired samples from 107 patients were used in this study
- Patient demographics were as follows:
  - Median age was 58 years and 32% of patients were ≥ 65 years of age
  - 57% of patients were female
  - 55% of patients were white, 5% were black, 20% were Asian, 5% were another race. Data on race were not available for 16% of patients

Assessment of *FLT3*-ITD in BM and PB

- In 97.2% of samples (104/107 patients), *FLT3*-ITD mutation was detected in both BM and PB, or in neither sample
  - 82.2% (88/107 patients) had *FLT3*-ITD mutations in both BM and PB
  - 14.9% (16/107 patients) had *FLT3*-ITD mutations in neither BM or PB
- 2.8% (3/107 patients) had *FLT3*-ITD mutation detected only in BM with no mutation detected in paired PB sample, despite the presence of circulating blasts

Blast Count and Presence of ITD

- No correlation between blast count and presence of ITD was observed (**Table 1**)
- Some patients with a low blast count had ITD in BM and/or PB
  - Some patients with no detectable blasts in PB still had detectable *FLT3*-ITD in PB
- There were 2 cases where an additional ITD was detected in BM or PB, but the major ITD was present in both specimen types
- In patients with no ITD detected in either specimen type, blast counts ranged from 3% to 67% in BM and 2% to 54% in PB

Table 1. Lack of Correlation Between Blast Count and Length or Ratio of ITD in Bone Marrow and Peripheral Blood in Representative Patients

	Specimen type	Blasts, %	ITD1 size, bases	ITD1 ratio, %	ITD2 size, bases	ITD2 ratio, %
Patient A	BM	85	84	95	ND	
	PB	93	84	93	54	1
Patient B	BM	ND	78	22	69	1
	PB	6	78	18	ND	
Patient C	BM	33	54	21	ND	
	PB	4	54	6	ND	

A second ITD was present in PB or BM of some patients, but the larger ITD was present in both specimen types.
Abbreviations: BM, bone marrow; ITD, internal tandem duplication; PB, peripheral blood; ND, not detected.

Length and Number of ITDs

- In 88 patients with *FLT3*-ITD in both BM and PB, the length of ITD was between 9 and 198 bases
  - In all cases, length of the ITD was identical in BM and PB
  - Across a wide range of ITD sizes, agreement of ITD size between specimen types was observed (**Table 2**)
  - 23.9% of patients (21/88) had multiple ITDs
    - Multiple ITDs were detected in both specimen types for most of these patients
    - 2 patients exhibited 3 distinct ITDs

Allelic Ratio

- Across all samples with ITD, the ITD allelic ratio ranged from 1% to 96%
  - The ratio was similar or slightly lower in PB compared to BM (**Table 2**)
  - In 26% (23/88) cases, the allelic ratio in the PB was lower than that in the BM by ≥ 10%
  - No correlation between blast count and allelic ratio was found in either specimen type

Table 2. Length and Ratio of ITD in Bone Marrow and Peripheral Blood in Representative Patients

	Specimen type	Blasts, %	ITD1 size, bases	ITD1 ratio, %	ITD2 size, bases	ITD2 ratio, %
Patient D	BM	86	42	82	15	1
	PB	87	42	71	15	1
Patient E	BM	29	27	21	18	2
	PB	45	27	23	18	1
Patient F	BM	90	87	87	66	2
	PB	91	87	71	66	5
Patient G	BM	84	186	92	147	1
	PB	61	186	95	147	1
Patient H	BM	ND	198	35	ND	
	PB	39	198	41	ND	

Abbreviations: BM, bone marrow; ITD, internal tandem duplication; PB, peripheral blood; ND, not detected.

Equivalency Study Statistics

Deming Regression (Figure 2)

- Good correlation was observed between specimen types
- $y = 1.05x–7.54$; $R^2 = 0.866$, $S_{y|x} = 11.7$. Slope 95% CI, 0.98-1.12

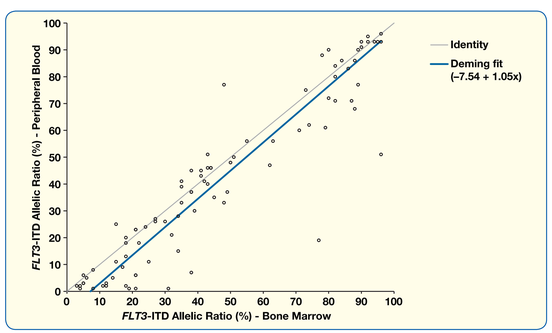

Figure 2. Scatter plot with Deming fit.

Bland-Altman Difference Plot (Figure 3)

- Good agreement between specimen types was observed
- Good random scatter around average bias of –5.2% with 95% CI including 0.0% (95% CI, –27.6-17.2)

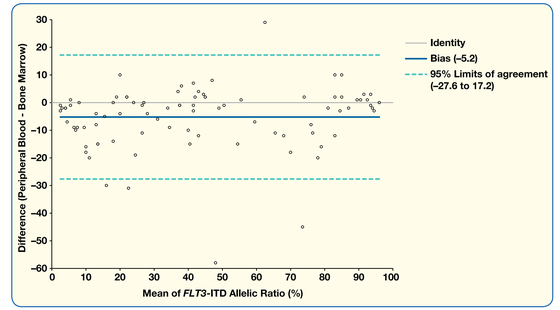

Figure 3. Bland-Altman difference plot.

CONCLUSIONS

- All newly-diagnosed AML patients are tested for mutations. This assessment is currently performed on BM aspirate or biopsy based on the common belief that PB is not adequate for this assessment
- We analyzed paired BM and PB samples from more than 100 patients with relapsed or refractory AML for *FLT3*-ITD mutations. Our results show a high degree of concordance between PB and BM specimens for assessment of *FLT3*-ITD mutations
  - 97.2% of patients showed agreement in *FLT3*-ITD mutational status between BM and PB
  - The length of ITDs agreed between BM and PB in all cases
  - The allelic ratio of *FLT3*-ITDs was similar between BM and PB in most paired patient specimens, showed good correlation in Deming Regression analysis, and good agreement when using Bland-Altman difference plots
  - We recommend that concordance between BM and PB specimens should be confirmed in patients with newly diagnosed AML
- No correlation between blast count and presence of *FLT3*-ITD was observed in either specimen type
- As FLT3 inhibitors are developed for clinical use, regular monitoring of patients' residual disease burden/effectiveness of therapy through assessment of *FLT3* status may become an important element of monitoring therapeutic responses; thus, being able to use peripheral blood samples for testing is beneficial to the patient

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Conflict of Interest Disclosures

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