Feasibility of Peripheral Blood Sampling and Correlation Between Early and Late Responses in Patients With Philadelphia Chromosome-Positive Chronic Myeloid Leukemia in Chronic Phase: Post-Hoc Analyses of Molecular Monitoring of Imatinib Response in the RIGHT Study

Luke P. Akard,[1] Jorge E. Cortes,[2] Maher Albitar,[3] Stuart L. Goldberg,[4] Ghulam Warsi,[5] Meir Wetzler,[6] Solveig G. Ericson,[5] Jerald P. Radich[7]

[1]Indiana Blood and Marrow Transplantation, St. Francis Hospital and Health Centers, Indianapolis, IN, USA; [2]The University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; [3]Neogenomics Laboratories, Irvine, CA, USA; [4]John Theurer Cancer Center of Hackensack University Medical Center, Hackensack, NJ, USA; [5]Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA; [6]Roswell Park Cancer Institute, Buffalo, NY, USA; [7]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

INTRODUCTION

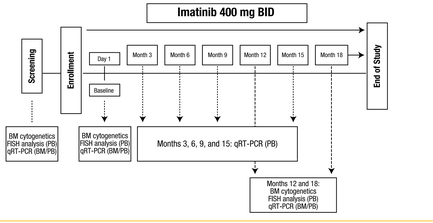
- The RIGHT (Rationale and Insight for Gleevec High-dose Therapy) study evaluated the effect of imatinib 400 mg BID on achievement of molecular and cytogenetic responses up to 18 months after therapy initiation in 115 patients with newly diagnosed chronic myeloid leukemia (CML) in chronic phase (CP).[1]
- The RIGHT study demonstrated rapid achievement of major molecular response (MMR) and complete molecular response (CMR) with imatinib treatment: 6 months, 48% and 39%; 12 months, 54% and 44%; 18 months, 63% and 55%, respectively.[1]
- Monitoring achievement of response milestones is important for the successful management of CML, since there is growing evidence demonstrating a significant positive correlation of early response with long-term outcomes.[2]
- Frequent guideline-recommended response monitoring has generated interest in less invasive testing methods.
- Recent studies in patients receiving BCR-ABL1 TKIs have suggested an excellent correlation between peripheral blood (PB) or bone marrow (BM) FISH and PB and BM quantitative reverse transcription polymerase chain reaction (qRT-PCR).[3,4]
- The post-hoc analyses of the RIGHT study presented here sought to determine:
  - Strength of correlation between molecular analysis data by qRT-PCR using PB or BM samples versus cytogenetic analysis data by PB FISH or chromosome analysis
    - Analyses were based on multiple corresponding samples.
  - Whether early molecular response predicts future molecular response

STUDY OBJECTIVES

- The objectives of the study were to determine correlation between:
  - Cytogenetic response as assessed by FISH and by chromosomal analysis
  - Cytogenetic response as assessed by FISH and *BCR-ABL1* log reduction as assessed by qRT-PCR
  - *BCR-ABL1* log reduction at 3, 6, and 9 months and rate of MMR at 18 months

METHODS

Figure 1. Study Design



BM, bone marrow; FISH, fluorescence in situ hybridization; PB, peripheral blood; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction

Inclusion/Exclusion Criteria

- ≥ 18 years of age, diagnosed with CML-CP within 6 months, previously untreated or treated with imatinib for ≤ 1 month
- Adequate end organ function
- ECOG performance status 0-2
- No other clinically significant primary malignancy
- No severe and/or uncontrolled medical disease
- No grade III/IV cardiac disorders (NYHA criteria)

ECOG, Eastern Cooperative Oncology Group; NYHA, New York Heart Association

Laboratory Testing

- Hematologic (neutrophil and platelet counts), cytogenetic (BM and PB chromosomal analysis, and PB FISH), and molecular (qRT-PCR) testing were carried out at a single commercial reference laboratory.
  - Molecular testing by qRT-PCR was performed by Quest Diagnostics and validated at Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA, USA.
- Adequate sampling for cytogenetic analysis required ≥ 20 metaphases.
- Samples for correlation analyses were taken on the same date.

Molecular Response

- The baseline ratio of *BCR-ABL1:ABL1* (standardized baseline) was calculated at Quest Diagnostics from all prestudy samples in this trial in addition to other diagnostic samples assayed in this laboratory.
- Log reduction in *BCR-ABL1* transcripts was calculated as the change from standardized baseline value (not individual patient baseline) of 3.7970 in log-transformed (base 10) *BCR-ABL1:ABL1* transcript ratio (per international scale).
- MMR was defined as ≥ 3-log reduction in *BCR-ABL1* transcript levels from standardized baseline value validated by the FHCRC.

Statistical Methods

- Spearman's rank order correlation was used for all correlations.
- The *P* values are from a test of the null hypothesis that the correlation is zero against the alternative hypothesis that it is non-zero.

RESULTS

Table 1. Baseline Characteristics[1]

Characteristics (N = 115)	No.	%
Median age (range), years	50 (19-81)	
< 65	91	79
≥ 65	24	21
Sokal risk score		
Low	80	70
Intermediate	20	17
High	14	12
Missing	1	1
Previously treated with imatinib*	19	17
Median duration of imatinib treatment (range), days	19 (1-38)	
Median time from diagnosis to first study dose (range), months	0.96 (0.13-9.69)	
Clonal evolution†	3	3

*Received imatinib for < 1 month per protocol inclusion criteria.
†Clonal evolution is defined as the presence of other chromosomal abnormalities in addition to Philadelphia chromosome.

- There were statistically significant pairwise correlations between FISH, chromosome analysis (karyotype), qRT-PCR with PB, and qRT-PCR with BM (Table 2).

Table 2. Pairwise Overall Correlation Among Chromosome Analysis, FISH, qRT-PCR (PB), and qRT-PCR (BM)

Pairwise comparators		No. of samples	Correlation coefficient	*P* value
FISH	Chromosome analysis	151	0.9198	$<10^{-4}$
FISH	qRT-PCR (BM)	181	0.8583	$<10^{-4}$
FISH	qRT-PCR (PB)	352	0.8729	$<10^{-4}$
qRT-PCR (BM)	Chromosome analysis	193	0.8054	$<10^{-4}$
qRT-PCR (BM)	qRT-PCR (PB)	170	0.9256	$<10^{-4}$
qRT-PCR (PB)	Chromosome analysis	126	0.8193	$<10^{-4}$

- At 12 months, cytogenetic response as assessed by chromosome analysis correlated well with cytogenetic response as assessed by FISH (Table 3).

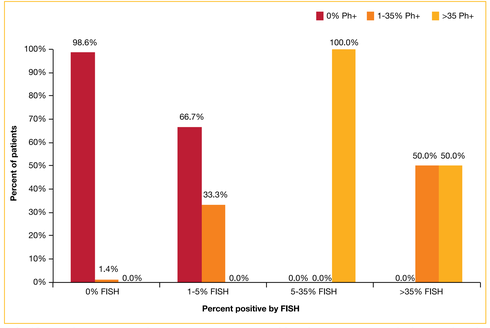
Table 3. Comparison of 12-month Cytogenetic Response by Chromosome Analysis and FISH

		Chromosome analysis		
		0% Ph+	1-35% Ph+	>35% Ph+
FISH	0% FISH+	72	1	0
	1-5% FISH+	4	2	0
	5-35% FISH+	0	0	2
	>35% FISH+	0	1	1

Note: 90 patients had FISH analysis and 83 had BM chromosome analysis performed at 12 months; the 7 patients missing chromosomal analysis data all were 0% FISH+.

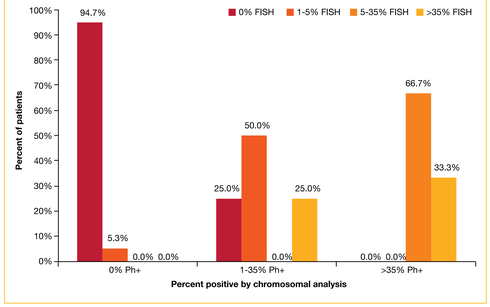
- At 12 months, cytogenetic response as assessed by FISH correlated well with cytogenetic response as assessed by chromosome analysis (Figure 2a).

Figure 2a. Correlation of Cytogenetic Response at 12 Months by Chromosomal Analysis vs FISH



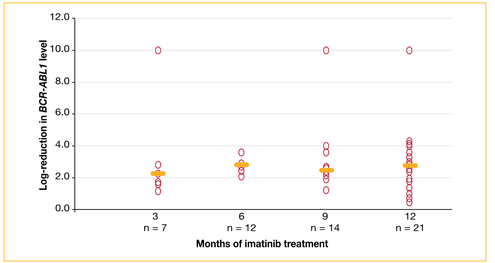
- At 12 months, cytogenetic response as assessed by chromosome analysis correlated well with cytogenetic response as assessed by FISH (Figure 2b).

Figure 2b. Correlation of Cytogenetic Response at 12 Months by FISH vs Chromosomal Analysis



- Log reduction in *BCR-ABL1* transcripts by qRT-PCR correlates with cytogenetic response as assessed by FISH (Figure 3).
- The median log reduction in *BCR-ABL1* transcripts from baseline to time of first documentation of FISH-negative status was 2.7 logs.

Figure 3. Log Reduction in *BCR-ABL1* Level at the Time of First Documentation of FISH-negative Status



Note: One patient had first documentation of FISH-negative status at 18 months; log-reduction in BCR-ABL1 level was 3.5794 at that time

- Molecular response at 3, 6, and 9 months correlated with achievement of MMR at 18 months (Table 4).

Table 4. Log Improvement in *BCR-ABL1* Level From Baseline at 3, 6, and 9 Months and MMR at 18 Months

Time on imatinib	Log improvement	No. of patients with log improvement	No. (%) of patients with MMR at 18 months
3 months (n = 81*)	< 1 log	9	3 (33)
	1-2 log	22	12 (55)
	> 2 log	50	40 (80)
6 months (n = 78*)	< 1 log	6	0 (0)
	1-2 log	3	2 (67)
	> 2 log	69	52 (75)
9 months (n = 76*)	< 1 log	5	0 (0)
	1-2 log	8	3 (38)
	> 2 log	66	50 (76)

*Number of evaluable patients at each timepoint; for non-evaluable patients, samples were either not collected or not suitable for analysis.

- Molecular response at 3, 6, and 9 months correlated with achievement of CCyR at 18 months (Table 5).

Table 5. Log Improvement in *BCR-ABL1* Level From Baseline at 3, 6, and 9 Months and CCyR at 18 Months

Time on imatinib	Log improvement	No. of patients with log improvement	No. (%) of patients with CCYR at 18 months
3 months (n = 83*)	< 1 log	9	4 (44)
	1-2 log	24	20 (83)
	> 2 log	50	45 (90)
6 months (n = 79*)	< 1 log	6	2 (33)
	1-2 log	3	2 (67)
	> 2 log	70	62 (89)
9 months (n = 81*)	< 1 log	5	0 (0)
	1-2 log	8	4 (50)
	> 2 log	68	63 (93)

*Number of evaluable patients at each timepoint; for non-evaluable patients, samples were either not collected or not suitable for analysis.

Study Limitations

- Because FISH testing was done at 3-month intervals, the exact time of FISH conversion is not known. It is likely that the log-reduction in *BCR-ABL1* level would be greater if the exact time of FISH conversion to negative status were known.
- Since this was an 18-month study looking at initial response, the ability to demonstrate the impact of early response on ultimate outcome and survival is limited.

CONCLUSIONS

- **Pairwise correlations between chromosome analysis of Ph+ status, FISH determination of Ph+ status, and qRT-PCR quantitation of *BCR-ABL1* transcripts using BM and PB were strong and statistically significant.**
- **Quantitation by qRT-PCR using PB correlated well with that using BM.**
- **A median reduction in *BCR-ABL1* transcripts of 2.7 logs (from a standardized baseline value of 3.7970) corresponded to FISH-negative status.**
- **Early reduction in *BCR-ABL1* transcripts correlated with achievement of MMR and CCyR at 18 months. Early response is consistent with improved long-term outcomes.**

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