BCR-ABL1 MOLECULAR RESPONSES AT 12-18 MONTHS PREDICT LONG-TERM EVENT-FREE SURVIVAL IN PATIENTS WITH TYROSINE KINASE INHIBITOR (TKI)-TREATED CHRONIC MYELOGENOUS LEUKEMIA (CML)

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SUMMARY

- A multi-center clinical outcome evaluation for determining event-free survival (EFS) at 32-40 months was performed on chronic phase CML patients using the QuantideX® qPCR BCR-ABL IS Kit at 12-18 months after start of TKI therapy.
- Kaplan-Meier survival curves demonstrated a 22% increase of EFS rate at 3 years between groups above / below MR3 (p=0.028).
- Analytical reproducibility panels of RNA diluted from 10 to 0.01% IS showed %CV values of 14-30%.
- Test results are expressed as the Molecular Reduction (MR value) from the baseline of 100%IS (International Scale).

INTRODUCTION

To reaffirm the clinical utility of *BCR-ABL1* monitoring in patients with TKI-treated CML, a correlation between molecular response values and long-term outcome was determined. The QuantideX qPCR BCR-ABL IS Kit is a nucleic acid amplification test for the quantitation of *BCR-ABL1* RNA in human white blood cells enriched from EDTA whole blood.

The test uses standard hydrolysis probe chemistry to quantitate *BCR-ABL1* and the *ABL1* reference gene RNAs. Associated software reports an international scale (IS) *BCR-ABL1* value and a log-transformed MR value.

The kit contains:

- Reagents for amplification of *BCR-ABL1* major breakpoints (e13a2 and e14a2)
- Reagents for amplification of endogenous control gene (*ABL1*)

MATERIALS AND METHODS

Primary objective: establish event-free survival in patients with MR≥3 vs MR<3 using the Kaplan-Meier survival function.

Clinical testing: 3 laboratories performed *BCR-ABL1* testing on a total of 137 samples from 96 chronic phase CML patients' banked RNA specimens.

- Specimens were drawn 12-18 months after starting on TKI therapy. For subjects with 2 specimens between 12 and 18 months the first specimen was used towards this primary objective (Table 1) and both were used towards the standard error calculations in a secondary analysis (Table 2)
- Clinical events (TKI therapy change, loss of complete hematologic or cytogenetic response, progression to accelerated phase/blast crisis, kinase domain mutation, or death) were recorded through 36±4 months after starting the TKI therapy

Reproducibility testing: 2 operators per site tested samples ranging from MR1.0-MR4.0 in multiple replicates over 5 days.

Research funded by Asuragen.
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Presented at AMP 2016 - H54

RESULTS

The BCR-ABL RNA assay demonstrated the following overall performance characteristics at 36 months:

Event-free Survival Difference	SE	Z-score	P-value	Lower 95% Confidence Interval	Upper 95% Confidence Interval
22.2%	10.1%	2.20	0.0279	2.0%	42.4%

Table 1: Overall performance characteristics – primary endpoint, all subjects (n=96).

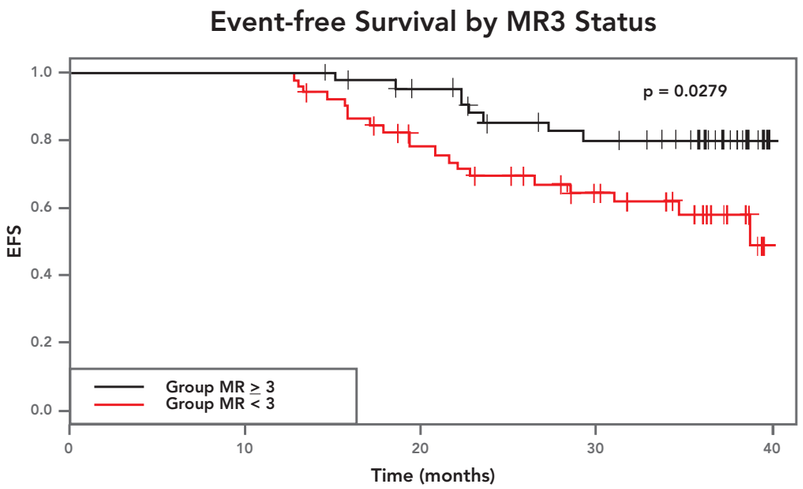

Figure 1. Survival curves of all subjects by MR3 status (n=96).

The difference in event-free survival rate between subjects above and below MR3.0 exceeded 10 percentage points and was significantly different from zero at the one-sided 2.5% level which demonstrated success in meeting the primary study objective.

Table 2 shows the event-free survival rate by MR3 status among subjects with known event status.

EFS Rate at 36 months, MR≥3	EFS Rate at 36 months, MR<3	Difference	Lower 95% Confidence Interval	Upper 95% Confidence Interval
77.8%	45.9%	31.8%	9.5%	50.1%

Table 2. Subjects with known 36-month event status by MR3 status (n=73).

Of the 51 subjects with MR<3.0 (not achieving MMR) at 12-18 months post-TKI:

- 20 had a clinical event
- 17 had no event
- 14 were lost to follow-up

Of the 45 subjects with MR≥3.0 (achieving MMR) at 12-18 months post-TKI:

- 8 had a clinical event
- 28 had no event
- 9 were lost to follow-up

Kaplan-Meier survival curves demonstrated a 22% increase of event-free survival rate (95% CI 2-42%) at 36 months in the MMR (vs non-MMR) group, p=0.028.

- MR<3.0 36-month EFS = 58% (95% CI 44-75%)
- MR≥3.0 36-month EFS = 80% (95% CI 68-93%)

%IS			MR		
Target	Mean	%CV	Target	Mean	SD
10	17.9184	15.3	1	0.752	0.066
1	2.0655	18.5	2	1.692	0.080
0.1	0.1940	19.3	3	2.720	0.083
0.032	0.0597	29.9	3.5	3.243	0.127
0.01	0.0191	30.3	4	3.738	0.129

Table 3. Multi-site precision (reproducibility). Five pools (dilution series) with target MR values of 1, 2, 3, 3.5 and 4 were constructed with five samples in each pool (at each dilution level), for a total of 25 samples. Each sample was evaluated at three sites by two operators making quintuplicate measurements on two days for a total of 750 measurements. This dataset was analyzed using a nested random effect analysis of variance using the REML criterion in R version 3.2.2 and SAS PROC MIXED v9.3. The components estimated were: Site to site, Day to day within operator and site, and Within run (data not shown). The variance components for the samples were grouped by pool (i.e. across the 5 unique specimens at each target dilution level). Overall, the determination of MR values was reproducible within all variables tested (the maximum observed standard deviation was 0.099 for a single specimen at the MR4 target level). This supports the testing of specimens in singleton in the test.

CONCLUSIONS

- This retrospective study, conducted at 3 US clinical sites, establishes the clinical performance characteristics of the QuantideX qPCR BCR-ABL IS Kit on the ABI 7500 Fast Dx.
- Test demonstrated performance as assessed by ability to predict prolonged event-free survival by the endpoint 36 months after the initiation of TKI treatment as estimated by the Kaplan-Meier survival function.
- This BCR-ABL assay has excellent reproducibility and analytical sensitivity, and the achievement of MR>3 (major molecular response) by this assay predicts prolonged event-free survival in TKI-treated CML patients.
- The test kit allows sensitive detection of the MR4.5 cutoff that defines "complete molecular response" in ongoing treatment-free remission clinical trials.

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