New tool for monitoring molecular response in chronic myeloid leukemia

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Abstract

Background: Chronic myeloid leukemia (CML) treatment monitoring using PCR based peripheral blood testing provides improved test sensitivity over cytology but suffers from inadequate standardization due to variation inherent in the existing PCR methodologies. Standardized Nucleic Acid Quantification BCR-ABL (SNAQ-BCRABL) is a novel competitive template based peripheral blood b2a2/b3a2 transcript abundance method. It uses mixtures of b2a2 or b3a2 and GusB competitive templates and melting curve analysis to provide desired quality controls to correct for procedural variation. A pilot study was conducted in CML patients to evaluate the imprecision and linearity of two established real-time qPCR laboratory developed test (LDT) and SNAQ for monitoring BCR-ABL.

Method: Thirty six CML patients treated at our institution were enrolled in this pilot study. Their peripheral blood sample were analyzed at MD Anderson Molecular Diagnostic Laboratory and Cancer Genetics Institute for BCR-ABL by LDT (L1 and L2) and SNAQ (S1 and S2) test respectively. The LDT methods used TaqMan real-time assays to quantify BCR-ABL and ABL targets in cDNA synthesized from WBC RNA isolated from the blood specimens. The SNAQ method likewise used cDNA synthesized from WBC, but differs at the PCR step by the addition of known quantities of competitive templates (b2a2 or b3a2 and GusB) to the cDNA prior to PCR amplification and melting curve analysis. AccuGenomics's SNAQ software imports the melting curve data, performs quality control and curve fitting analysis to generate a BCR-ABL report. The LDT methods report %BCR-ABL/ABL results using International Standard, and the SNAQ results were reported as %BCR-ABL/GusB.

Results: Each test result (n= 36) was ranked against all the other samples tested by the same method. The Pearson correlation between SNAQ and LDT was met with correlations of 0.96, 0.96, 0.97 and 0.94 with L1 x S1, L1 x S2, L2 x S1 and L2 x S2 respectively. ANOVA of log %BCR-ABL interlaboratory results indicated a significant difference between LDT methods ($p<0.0000001$), but not with the SNAQ methods between labs ($p=0.98$) (Fig.1A).

Imprecision was estimated using the Bland-Altman method and the plot indicated that sample results from L2 had 1 outlier, which was excluded from analysis. All three L1 difference plots (Fig.1B & C) had a significant trend ($p<0.007$), requiring regression correction prior to estimating variation. The linear regression analysis, indicated L2 LDT was a large source of method bias.

Post hoc analysis of method agreement showed the SNAQ method did not generate any outliers and had a 95% limit of agreement of ±3-fold between laboratories, whereas L1 and L2 LDT method had significant differences despite reporting in international scale, with 95% limit of agreement of 2 to 20-fold.

Conclusion: In this pilot study, SNAQ methodology performed well suggesting it might be able to overcome some of the limitations encountered by some of the LDT currently in clinical practice. Additional studies with more patients and correlation with clinical outcomes are required to confirm this observation

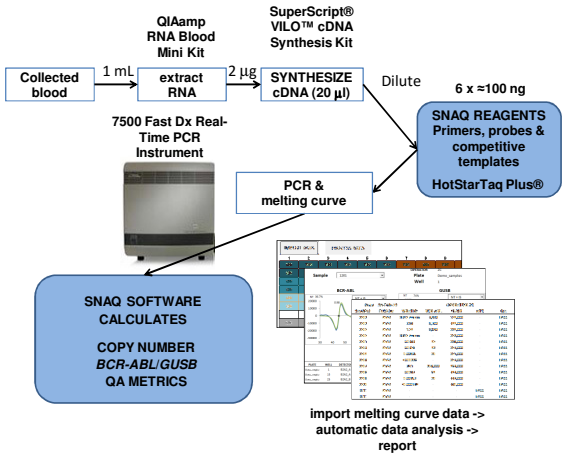
Background

- CML treatment monitoring using PCR based peripheral blood testing provides improved test sensitivity over cytology but suffers from inadequate standardization in most labs due to variations, inherent in the existing PCR methodologies.
- Variable results in most labs are probably due to different techniques, internal controls, reagents, and methods of calculation
- We present the initial analytic performance evaluation of a novel competitive template based peripheral blood b2a2/b3a2 transcript abundance method.
- Standardized Nucleic Acid Quantification BCR-ABL (SNAQ-BCRABL) uses mixtures of b2a2 or b3a2 and GusB competitive templates and melting curve analysis to provide clinically needed quality controls to correct for operator, instrument, sample, and reagent variation when measuring transcript abundance.
- We hypothesized that the SNAQ-BCRABL method produced more consistent results with less frequent laboratory to laboratory variability.

Methods

- Pilot study was conducted in CML patient to look at the imprecision and linearity of two established CLIA real-time qPCR assays and SNAQ-BCR ABL.
- Patients over 18 years of age, with known b2a2 or b3a2 allele of CML fusion, expected to be RT-qPCR positive for b2a2 and/or b3a2 (i.e., during their first year of CML therapy) were enrolled.
- Thirty six patients were enrolled in this pilot study. Blood was collected for RT-qPCR and SNAQ method on the same day to be tested at the MD Anderson molecular diagnostic laboratory and another laboratory in New Jersey for BCR-ABL evaluation by RT-qPCR and SNAQ method simultaneously.

SNAQ BCR-ABL work flow



- Blood is collected and shipped to laboratory for RNA extraction and cDNA synthesis.
- Copy DNA is divided into three mixtures of internal standard (MIS A, B & C), *GUSB* & *BCR-ABL* primer/probes & master mix (100 ng/ PCR).
- Samples are amplified and melting curve data collected and exported to a spreadsheet where melt curves are fit to a hybridization model to generate a native template : internal standard ratio and QA metrics (sample load, product specificity).
- This ratio is used to calculate transcript abundance, from which *BCR-ABL*/*GUSB* ratio is calculated.

Test Results

Sample #	LAB 1 qRT-PCR	LAB 2 qRT-PCR	LAB 1 SNAQ	LAB 2 SNAQ
1	0.039	0.143	0.055	0.076
2	Low pos	0.022	Low pos (=0.003)	Low pos (=0.007)
3	0.063	0.032	0.084	0.063
4	0.039	0.301	0.140	0.159
5	0.763	0.004	1.031	1.324
6	0.406	5.149	0.406	1.224
7	Low pos	0.058	0.010	Low pos (=0.006)
8	8.036	37.649	9.777	7.706
9	Undetectable	Undetectable	<0.0053	<0.0066
10	0.042	0.382	N/A	0.118
11	0.196	0.968	0.387	0.185
12	18.459	27.981	12.789	11.342
13	0.987	3.893	0.618	0.718
14	0.014	0.111	0.042	0.027
15	4.183	23.497	7.017	3.310
16	0.018	0.154	0.020	0.054
17	0.200	3.007	0.659	0.591
18	0.144	2.935	0.649	0.827
19	18.914	72.291	14.863	15.319
20	0.070	0.331	0.111	0.052
21	Undetectable	Undetectable	<0.0066	<0.0047
22	Undetectable	Undetectable	<0.0174	<0.008
23	0.130	1.915	0.228	0.184
24	0.088	1.011	0.285	0.177
25	0.007	0.078	0.030	0.018
26	Low pos	0.049	<0.0103	0.011
27	Undetectable	0.005	<0.0064	<0.0051
28	Low positive	Undetectable	<0.0018	<0.0111
29	Undetectable	0.108	0.008	0.021
30	Undetectable	Undetectable	<0.0063	<0.0033
31	0.014	0.175	0.018	0.031
32	0.676	4.051	1.914	1.024
33	0.011	0.184	0.032	0.069
34	0.074	0.860	0.134	0.274
35	2.254	4.779	1.384	0.910
36	3.591	Undetectable	1.086	0.873
37	0.084	0.456	0.146	0.136
38	0.077	0.402	0.156	0.121
39	0.025	0.378	0.094	0.070

qRT-PCR; quantitative real time polymerase chain reaction, SNAQ; standardized nucleic acid quantification test, Undetectable; BCR-ABL1 fusion transcripts negative , Low pos; low positive, unable to quantify.

Interlaboratory Correlation

(a) Pearson correlation

	1L	2L	1S	2S
1L	1.00	0.93	*0.97*	*0.96*
2L		1.00	*0.94*	*0.96*
1S			1.00	0.96
2S				1.00

1L; lab 1 LDT, 2L; lab 2 LDT, 1S; lab 1 SNAQ, 2S; lab2 SNAQ

The primary study goal required a >0.85 correlation between SNAQ and LDT was met with correlations of 0.96, 0.96, 0.97 and 0.94 with 1Lx2S, 2Lx2S, 1Sx1L and 2Sx2L respectively.

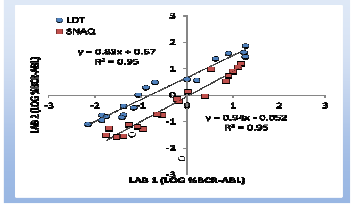
(b) Kendal Tau interlaboratory correlation

	1L	2L	1S	2S
1L	1.00	0.86	*0.91*	*0.87*
2L		1.00	*0.86*	*0.90*
1S			1.00	0.88
2S				1.00

1L; lab 1 LDT, 2L; lab 2 LDT, 1S; lab 1 SNAQ, 2S; lab2 SNAQ

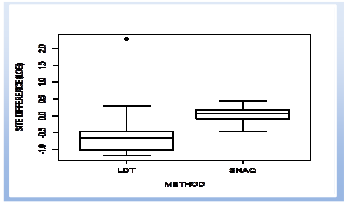
- The SNAQ methods correlation between lab 1 and 2 was slightly better (1Sx2S, 0.88) than the LDT between two labs (1Lx2L, 0.86).
- The primary study goal of a >0.85 SNAQ correlation with the LDT was met; 0.91, 0.87, 0.86 and 0.90 in 1Sx1L, 1Lx2S, 2Lx1S and 2Sx2L respectively.

Interlaboratory Log % BCR-ABL result correlation plot



Correlation comparison by method type real-time qPCR (circles) or SNAQ (squares). Two outliers in LDT methods from Lab 2.

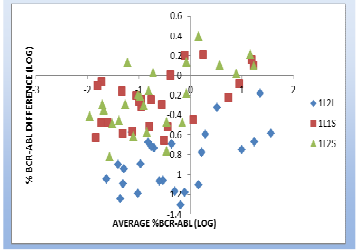
Analytical accuracy estimate



The average fold difference for LDT was 7.1 +/-4.4 fold, which corresponds to an accuracy of 16-fold. The SNAQ method had and average of 1.24 +/-0.62, 18-fold, which corresponds to a 2.5-fold accuracy.

*Accuracy = average diff + 2 x SD diff

Bland-Altman plot of % BCR-ABL response



- For each sample with quantitative results by all four methods, the %BCR-ABL log difference between the indicated methods (1L, Lab 1 LDT; 2L, Lab 2 LDT; 1S, Lab 1 SNAQ; 2S, Lab 2 SNAQ) were plotted vs. their average log %BCR-ABL

Conclusions

- SNAQ BCR-ABL pilot study met its primary concordance goal with established CLIA BCR-ABL monitoring tests.
- Post hoc analysis of method agreement showed the SNAQ method did not generate any outliers and had a 95% limit of agreement of ±3-fold between laboratories.
- The two LDT methods had significant differences despite reporting in international scale, with 95% limit of agreement of 2 to 20-fold.
- Additional studies with more patients and correlation with clinical outcomes are required to confirm this observation.

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