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TaqMan RT-PCR assay coupled with capillary electrophoresis for quantification and identification of *bcr-abl* transcript type

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Chronic myelogenous leukemia is characterized by the presence of the reciprocal t(9;22)(q34;q11) in which c-*abl* located on chromosome 9, and the *bcr* locus located on chromosome 22, are disrupted and translocated creating a novel *bcr-abl* fusion gene residing on the derivative chromosome 22. In most cases, the breakpoint in *abl* occurs within intron 1. Depending on the breakpoint in *bcr,* exon 2 of *abl* (a2) joins with exons 1 (e1), 13 (b2), or 14 (b3), or rarely to exon 19 (e19) of *bcr* resulting in chimeric proteins of p190, p210 and p230, respectively. Currently, several multiplex real-time reverse transcriptase-polymerase chain reaction (RT-PCR)-based assays for detecting *bcr-abl* are available to assess the levels of the three common fusion transcripts, b2a2, b3a2 and e1a2. Although these assays circumvent the requirement for individual fusion sequence quantitative polymerase chain reaction-based assays, they do not identify the specific fusion transcript. Knowledge of the latter is useful to rule out false-positive results and to compare clones before and after therapy. We designed a novel multiplex real-time RT-PCR assay to detect *bcr-abl* that allows accurate quantification and determination of the specific fusion transcript. In this assay, *abl* primer labeled at its 5′ end with the fluorescent dye NED (Applied Biosystems) is incorporated into the *bcr-abl* fusion product during amplification. The NED fluorescent dye in *abl* primer, without interfering with fluorescent TaqMan probe signal, allows subsequent identification of the fusion transcript by semiautomated high-resolution capillary electrophoresis and GeneScan analysis.
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The hallmark of chronic myelogenous leukemia is the t(9;22)(q34;q11). In this translocation, the 3′ segment of the c-*abl* proto-oncogene on chromosome 9 is juxtaposed with the 5′ segment of the *bcr* gene on chromosome 22.[1–5] Breaks in the c-*abl* gene typically occur in the first intron. Breaks in *bcr* generally occur in one of three regions: the major breakpoint cluster region (M-*bcr*), the minor breakpoint cluster region (m-*bcr*) and the micro breakpoint cluster region (μ-*bcr*).[6] Breakpoints occurring in M-*bcr* involve introns 13 or 14 and join exon 13 (also known as b2) or 14 (also known as b3) with exon 2 of *abl* (a2) resulting in the fusion transcripts b2a2 and b3a2, respectively. These transcripts lead to the production of an 8.5 kb transcript coding for a 210 kDa (p210) chimeric protein.[7,8] Breakpoints in m-*bcr* involve the first intron of *bcr* and join exon 1 (e1) with a2 resulting in a smaller fusion transcript, e1a2, that codes for a 190 kDa (p190) protein.[9] Breakpoints in μ-*bcr* involve intron 19 and result in the joining of exon 19 (e19) of *bcr* with a2, e19a2, coding for a 230 kDa (p230) protein.[10] Although all *bcr-abl* fusion proteins display activated tyrosine kinase activity, the p190 form has been shown to have more transforming potential than p210 in *vitro* and in *vivo*.[11–13] Fusion transcripts b3a2 and b2a2 account for the majority of CML cases. The e1a2 fusion transcript is seen primarily in t(9;22)-positive acute lymphoblastic leukemia and lymphoid blast phase of chronic myelogenous leukemia, but rarely in chronic myelogenous leukemia in chronic phase. The rare e19a2 fusion transcript is found in cases of chronic myelogenous leukemia with prominent neutrophilic maturation.[14] These unusual neoplasms also have been reported in the literature

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by some investigators as chronic neutrophilic leukemia.[15]

The introduction of fluorescence-based PCR methods has greatly simplified detection and quantification of *bcr-abl* fusion transcripts. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) methods enable t(9;22) detection and quantification without the need to open amplification tubes and are target specific, highly sensitive and reproducible. Hence, the real-time RT-PCR approach has become the preferred method for monitoring tumor burden and the effect of therapy in patients with t(9;22)-positive leukemias.[16–24] Two strategies are generally followed to quantify the common of b2a2, b3a2 and e1a2 fusion transcripts. In one approach, a multiplex qualitative PCR is performed initially. Following gel electrophoresis and identification of the specific fusion transcript, real-time RT-PCR is performed using fusion sequence specific primers to measure *bcr-abl* transcript levels. In the second approach, a multiplex real-time RT-PCR assay that detects the three fusion transcripts is performed directly, without prior analysis or determination of the fusion transcript type. The first approach involves two independent PCR assays, is time consuming and is prone to manual errors and contamination. Using the second approach, one cannot determine the specific fusion transcript present in the sample unless real-time RT-PCR products are subjected to gel electrophoresis and ethidium bromide-based detection, a comparatively less-sensitive technique that is not very useful for monitoring minimal residual disease. A third strategy can also be pursued, that being two (or multiple) separate real time RT-PCR assays for each transcript type separately. Although this approach allows for easy identification of the specific transcripts, it basically multiplies the workload of the second approach.

In a previous study,[25] we showed that PCR products labeled during real-time PCR by incorporation of a fluorescent dye-labeled primer can be resolved by capillary electrophoresis and GeneScan analysis in follicular lymphoma cases with the t(14;18). We thought this approach could be applied to analysis of *bcr-abl* transcripts in patients with t(9;22)-positive leukemias. In this assay, we labeled the 5′ end of the *abl* primer with the fluorescent dye NED (Applied Biosystems), which is included along with e1 and b2 *bcr* primers during the TaqMan RT-PCR assay and thus is incorporated into the *bcr-abl* fusion product. This allows specific identification of the fusion transcript type by capillary electrophoresis equipped with highly sensitive laser-based detection of the fluorescently labeled fusion products. This approach, which has a sensitivity of detection equivalent to other real-time RT-PCR assays, requires no further manipulation to confirm or identify the specific fusion transcript in patient specimens.

Materials and methods

Patient Specimens and Controls

A total of 67 bone marrow (BM) aspirate specimens from patients with *bcr-abl*-positive leukemias were included in this study. These specimens were divided into two groups. One group included 26 BM aspirates from patients selected randomly at various stages in their clinical course. Almost all of these patients were being treated for their disease with chemotherapy. The second group included 41 BM aspirates from nine patients assessed at time of diagnosis, prior to therapy, and then followed over time to assess response to therapy. The diagnosis of chronic myelogenous leukemia and acute lymphoblastic leukemia was based on standard clinical and pathologic criteria as stated in the World Health Organization classification[14] and used at The University of Texas MD Anderson Cancer Center. Only those patient samples that had *abl* transcript values greater than 20 000 copies by quantitative RT-PCR were included in the study. The *bcr-abl*-positive cell lines KBM7, K562 and B15 that carry b2a2, b3a2 and e1a2 fusion genes, respectively, served as positive controls. The HL60 cell line was used as a negative control. Serial dilutions of a plasmid containing *bcr-abl* fusion sequences[26] were analyzed simultaneously with patient samples to generate standard curves for *bcr-abl* and *abl* (normalizer). The standard curves for *bcr-abl* were similar whether the plasmid was diluted into water or into the HL60 cell line. Hence, plasmid diluted into water was used for construction of standard curves in order to calculate the quantities of *bcr-abl* and *abl* transcripts in a test sample.

For RNA isolation, total leukocytes were prepared by erythrocyte lysis of BM aspirate (1–2 ml) specimens collected in ethylenediaminetetraacetic acid (EDTA). Total RNA was isolated using Trizol reagent (Invitrogen Lifetechnologies Gaithersburg, MD, USA) according to the manufacturer's instructions. The integrity of RNA was determined by gel electrophoresis prior to reverse transcription. Total RNA ranging from 3 to 30 μg with intact 28 s and 18 s RNA was converted to cDNA using random hexamers and Superscript II reverse transcriptase (Invitrogen Lifetechnologies) according to the recommendations of the manufacturer. For specimens with less than 7 μg total RNA, the final reaction volume was adjusted to 15 μl, and for specimens with greater than 7 μg of total RNA the cDNA conversion was performed in 60 μl.

Real-time RT-PCR Assay for bcr-abl

Real-time RT-PCR assays were performed using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). A volume of 2.5 μl of cDNA from each sample was subjected to amplification in duplicate for *bcr-abl* in a multiplex

Table 1 Primers and probes used in the modified quantitative t(9;22) (q34;q11) RT-PCR

	Sequences
bcr-abl	
BCR-2F (b2)	5′-TGC AGA TGC TGA CCA ACT CG-3′
BCR-cF (e1)	5′-ACC GCA TGT TCC GGG ACA AAA-3′
ABL-N	5′-NED-TCC AAC GAG CGG CTT CAC-3′
bcr-abl TaqMan probe	5′-FAM-CAG TAG CAT CTG ACT TTG AGC CTC AGG GTC T-TAMRA-3′
Abl	
ABL2-F	5′-GTC TGA GTG AAG CCG CTC GT-3′
ABL2-R	5′-GGC CAC AAA ATC ATA CAG TGC A-3′
abl TaqMan probe	5′-VIC®-TGG ACC CAG TGA AAA TGA CCC CAA CC-TAMRA-3′

RT-PCR using an *abl* primer in combination with *bcr* b2- or e1-specific primers, and an *abl* probe labeled with 6-carboxyfluorescein (FAM) (Table 1). For size analysis of fusion transcripts by capillary electrophoresis, the *abl* primer was labeled with NED fluorescent dye at its 5′ end (ABL-NED). As a control, *abl* transcripts were amplified simultaneously in duplicate, but in a separate reaction, to confirm the quality of extracted RNA and to normalize *bcr-abl* values.

Both *bcr-abl* and *abl* PCR assays were performed in a final volume of 25 μl using 2.5 μl of cDNA and a universal master mix without uracil *N*-glycosylase (Applied Biosystems). *bcr-abl* amplification consisted of previously described primers BCR-2F, BCR-cF and ABL-N[27] at 400 nM each and 200 nM of FAM-labeled TaqMan *bcr-abl* probe. Amplification of *abl*, the normalizer, consisted 100 nM of each primer (ABL2-F and ABL2-R) and 200 nM VIC labeled TaqMan *abl* probe. Samples were subjected to 40 cycles of PCR, each cycle consisting of 95°C denaturation for 30 s, 57°C annealing for 20 s and 72°C extension for 45 s. The last cycle was followed by a 10-min elongation step at 72°C.

Data Analysis

The fluorescence emission data for each sample were analyzed immediately after PCR using Sequence Detection Software (SDS version 1.7, Applied Biosystems). The threshold cycle (Ct) values representing the PCR cycle number at which fluorescence signal is increased above an arbitrary threshold were exported into Microsoft Excel software for further analysis. The *bcr-abl* levels for each sample were expressed as a ratio of *bcr-abl* to *abl* $\times 100$.

GeneScan Analysis

Following real-time RT-PCR, each amplification product was diluted 10-fold with water and 0.5 μl the diluted products were then subjected to capillary electrophoresis in an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) with 16 capillaries. The 3100 Genetic Analyzer is a laser-based fluorescence detection system that automatically introduces the samples labeled with fluorescent dyes into a polymer-filled capillary for electrophoresis. We used the GenoTYPE-ROX 50-500 DNA ladder (Life Technologies, Gaithersburg, MD, USA) as internal size standards. The size of each amplified fragment is calculated with GeneScan software using the Local Southern sizing option (Applied Biosystems).

Results

Sensitivity of the Multiplex bcr-abl Real-time RT-PCR Assay

We assessed the effect of introducing *abl* primer labeled with the fluorescent dye NED on the sensitivity of multiplex TaqMan real-time RT-PCR *bcr-abl* assay by performing RT-PCR with the *abl*-NED primer and comparing the results with those obtained using an unlabeled *abl* primer. Serially diluted plasmid clone containing *bcr-abl* fusion sequences showed similar Ct values with and without NED labeled abl primer at all dilutions including 10 copies/assay, demonstrating that introduction of this fluorescent dye did not alter the efficiency or sensitivity of the *bcr-abl* assay (Table 2).

Interassay Variability

The interassay variability of the real-time quantitative RT-PCR for *bcr-abl* and *abl* was evaluated in 11 independent assays performed on different days over a period of 2 months. Serially diluted plasmid clone was used to generate standard curves for *abl* and *bcr-abl*. A constant amount of cDNA derived from the positive cell lines B15, KBM7 and K562 was also simultaneously amplified for *bcr-abl* and

Table 2 Correlation between traditional and modified real-time PCR assays for *bcr-abl*

	bcr-abl *Ct*	bcr-abl-*NED* *Ct*
Plasmid copy number		
100 000	19.41	19.72
10 000	23.10	23.04
1000	26.77	26.55
100	30.59	30.65
10	33.80	33.45
Cell line		
B15	25.42	25.67
KBM7	23.29	23.21
K562	26.19	26.35

Table 3 Interassay variability of the modified real-time t(9;22)(q34;q11) RT-PCR[a] and the real-time *abl PCR*

	bcr-abl			abl		
	Mean Ct	*s.d.*	*%CV*	*Mean Ct*	*s.d.*	*%CV*
Plasmid copy number						
100 000	19.51	0.46	2.33	19.59	0.61	3.14
10 000	23.14	0.42	1.82	23.03	0.56	2.44
1000	26.87	0.53	1.97	26.41	0.52	1.95
100	30.57	0.52	1.69	29.96	0.58	1.94
10	33.88	0.88	2.59	33.44	0.87	2.61
Cell line						
B15	25.51	0.81	3.16	20.98	0.99	4.7
KBM7	23.37	0.38	1.64	23.75	0.69	2.92
K562	26.08	1.64	2.49	25.23	0.78	3.09

[a]NED labeled abl primer was used for *bcr-abl* quantification as described in Materials and methods.

abl with each assay. To minimize variation, the same stock cDNA was used in all experiments. The mean, standard deviation (s.d.) and interassay coefficients of variation at each dilution of the plasmid and positive cell lines for *bcr-abl* and *abl* are shown in Table 3. S.d. of less than 1 Ct was observed with input plasmid copy number of 100 000, 10 000, 1000, 100 and 10. The standard curves plotted using mean threshold cycle (Ct) and mean copy number from 11 experiments for *bcr-abl* and *abl* indicate that the modified quantitative *bcr-abl* RT-PCR assay is linear over 5 logs and that amplification efficiencies for the two targets are similar (Figure 1a and b). The coefficient of variation in Ct values of positive control cell lines tested on 11 different days using a constant amount of cDNA each time varied from 1.64 to 3.16% for *bcr-abl* and 2.9 to 4.7% for *abl* (Table 3). These data indicate that the assay is reproducible and that 3–5-fold variation from experiment to experiment can be expected. Interestingly, the e1a2-positive B15 cell line consistently expressed a much lower level of *bcr-abl* fusion transcripts compared with *abl,* with an average *bcr-abl* to *abl* ratio of 2341/39127(0.06). The b2a2 and b3a2 positive cell lines, KBM7 and K562, expressed either similar or slightly higher levels of *bcr-abl* fusion transcripts compared with *abl*.

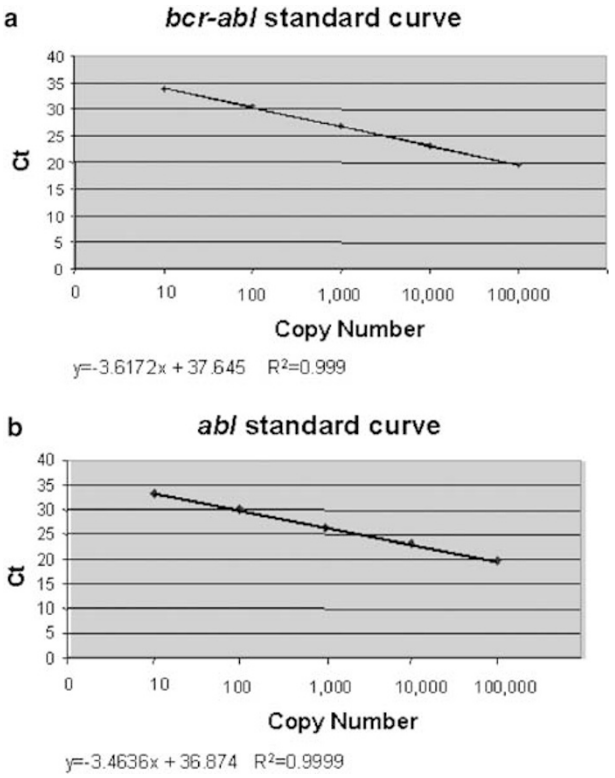

Figure 1 The standard curves plotted using mean threshold cycle (Ct) and mean copy number from 11 experiments for *bcr-abl* (**a**) and *abl* (**b**) indicate that the amplification efficiencies for the two targets are similar. (**a**) Also demonstrates that the modified quantitative t(9;22) RT-PCR assay including NED-labeled *abl* primer is linear over 5 logs.

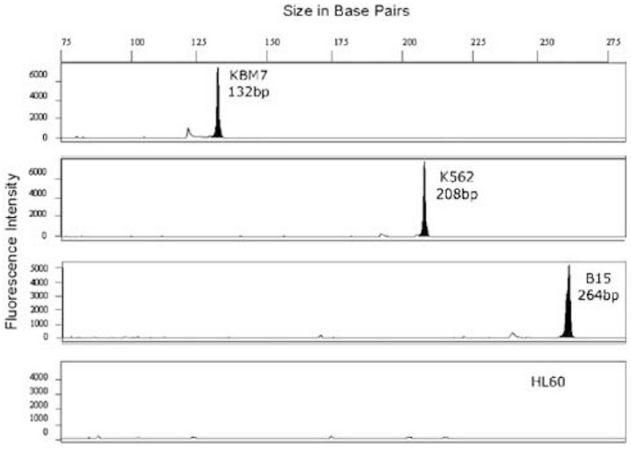

Figure 2 Electropherogram generated by GeneScan software shows the molecular size and fluorescence intensity of NED labeled amplification products generated by real-time PCR of t(9;22)-positive cell lines. Negative control, HL60 shows no peak (bottom panel), indicating the specificity of the assay.

Capillary Electrophoresis of Amplification Products

The amplification products were resolved by capillary electrophoresis and analyzed by GeneScan after real-time RT-PCR. Amplification of the positive control cell lines B15, KBM7 and K562 resulted in products of 264, 132 and 208 bp, respectively for the e1a2, b2a2 and b3a2 fusion transcripts using the primers described in Table 1. These results confirmed that fluorescent signal detected by real-time RT-PCR was due to the presence of fusion sequences (Figure 2). Amplification of the HL60 negative control showed no peak confirming the specificity of the assay. Amplification of serially diluted

plasmid standards and analysis of PCR products by capillary electrophoresis and GeneScan revealed that fusion transcripts could be identified even at 10 copies/assay (data not shown).

In order to determine if all three fusion sequences, when present in a sample at different levels, amplify uniformly and proportionately, we mixed cDNA synthesized using a constant amount of RNA from each of the positive cell line controls and performed RT-PCR as described in the methods. cDNA from the KBM7, K562 and B15 cell lines was mixed in the following ratios: 1:1:1; 1:0.1:1; 1:1:0.1 and 0.1:1:1. Thus, in the latter three mixing experiments, one transcript type was present at 10% of the amount of the other transcript types. The chromatograms generated by GeneScan analysis and shown in Figure 3 demonstrate that the three fusion sequences were amplified proportionately and that the RT-PCR assay allows detection of a fusion transcript present at lower levels compared with other fusion transcript types in the same sample.

Analysis of 26 randomly selected clinical specimens (Table 4) in patients with t(9;22)-positive chronic myelogeneous leukemia ($n=24$) and acute lymphoblastic leukemia ($n=2$) showed that the RT-PCR assay can detect a wide range of *bcr-abl* transcript copy numbers. Two untreated patients with chronic myelogenous leukemia had high copy numbers of b3a2 (case 23) and both b2a2 and b3a2 (case 24) transcripts, respectively. All other chronic myelogenous leukemia patients were being treated for variable intervals of time and had much lower *bcr-abl* transcript levels, or were completely negative. In positive samples, the lowest tumor burden detected was 0.0003 transcript copies (0.04%). Samples that were negative for *bcr-abl* by the RT-PCR assay were also negative by capillary electrophoresis and GeneScan analysis. All six patients with negative samples had no morphologic evidence of chronic myelogenous leukemia or cytogenetic evidence of the t(9;22) in the BM aspirate sample assessed by the RT-PCR assay. Two acute lymphoblastic leukemia patients (cases 25 and 26), both on therapy, had low levels of e1a2 transcripts. Thus, this approach is sensitive and reliable for identifying the specific fusion transcript in post-therapy samples and thereby allows monitoring of minimal residual disease.

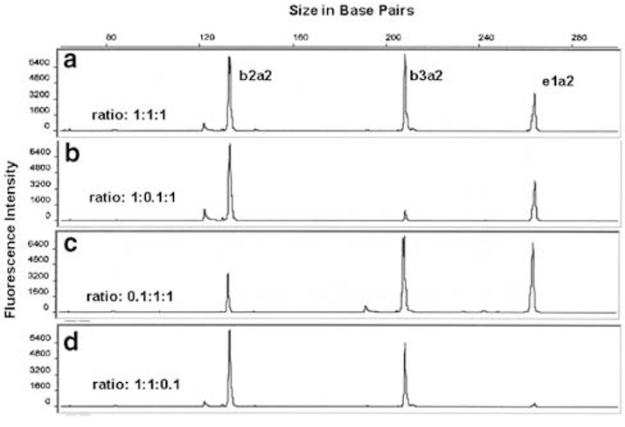

Figure 3 Cell line mixing experiments demonstrate that the multiplex real-time RT-PCR for *bcr-abl* allows proportionate amplification of the three transcripts when present at different levels in a sample. cDNA from KBM7, K562 and B15 was mixed in 1:1:1 ratio (**a**), 1:0.1:1 ratio (**b**), 1:1:0.1 ratio (**c**) and 0.1:1:1 ratio (**d**).

We then selected nine patients with t(9;22)-positive chronic myelogenous leukemia ($n=8$) or acute lymphoblastic leukemia ($n=1$) (Table 5) and assessed *bcr-abl* transcript type and *bcr-abl* copy number at time of diagnosis, before onset of therapy at our institution, and then assessed multiple follow-up samples obtained at 3, 6, 9 and 12 months on therapy. Eight patients (cases 1–8) had much higher transcript copy numbers at time of diagnosis than after treatment, with a dramatic drop off in levels within the first 3 months of therapy. In one patient (case 9), the patient had progressive disease with increasing levels of both b2a2 and b3a2 transcripts.

Discussion

In an earlier study,[25] we showed that PCR products labeled during real-time PCR by incorporation of a fluorescent dye-labeled primer can be resolved by capillary electrophoresis and GeneScan analysis in follicular lymphoma cases with the t(14;18). We thought that a similar approach to assess *bcr-abl* in patients with chronic myelogenous leukemia or acute lymphoblastic leukemia, either at time of diagnosis or for monitoring minimal residual disease, would be faster and more convenient compared with other approaches published in the literature.[16–23] For example, traditional quantitative real-time TaqMan RT-PCR assays used to assess for *bcr-abl* generally require prior knowledge of the type of fusion transcript present in a given specimen. Thus, a qualitative gel-based PCR is initially needed for this purpose. In contrast, most multiplex TaqMan *bcr-abl* RT-PCR assays reported previously do not require knowledge of the specific fusion transcript *a priori*, but one cannot distinguish which specific transcript is amplified. A third approach, that being assessment of each possible *bcr-abl* transcript by RT-PCR using multiple sets of specific primers, avoids the problems of traditional or previously published multiplex RT-PCR approaches, but is cumbersome.

In the assay we describe, labeling the *abl* primer at its 5′ end with NED, a fluorescent dye that does not interfere with TaqMan reporter dyes or assay sensitivity, allows easy identification of the specific fusion transcript after real-time PCR by automated high throughput capillary electrophoresis and GeneScan analysis. The latter also corroborates the quantitative PCR results and thus serves as another quality control for the real-time RT-PCR assay. For leukemia patients in whom two transcripts are

Table 4 Type of transcript and the number of *abl* and *bcr-abl* transcripts present in randomly selected follow-up specimens from patients diagnosed with chronic myelogenous leukemia[1–24] and acute lymphoblastic leukemia[25,26]

Patient	*Transcript type*	*Copies* bcr-abl	*Copies* abl	bcr-abl/abl $\times$ *100*
1	Negative	0	40 712	0
2	Negative	0	31 355	0
3	Negative	0	31 384	0
4	Negative	0	20 128	0
5	Negative	0	27 733	0
6	Negative	0	34 294	0
7	b3a2	0.13	46 374	0.0003
8	b3a2	1	24 889	0.004
9	b2a2 and b3a2	6	29 452	0.0204
10	b2a2	9	39 927	0.0225
11	b3a2	10	21 135	0.0473
12	b3a2	17	35 375	0.0481
13	b2a2 and b3a2	43	25 014	0.1719
14	b3a2	71	54 427	0.1304
15	b2a2	73	33 418	0.2184
16	b3a2	85	46 124	0.1843
17	b3a2	86	55 991	0.1536
18	b2a2	255	25 883	0.9852
19	b2a2	686	11 193	6.1288
20	b2a2	802	26 365	3.0419
22	b2a2 and b3a2	1127	19 662	5.7319
23	b3a2	5538	46 091	12.0154
24	b2a2 and b3a2	16 162	55 631	29.0521
25	e1a2	64	176 000	0.0364
26	e1a2	344	112 000	0.3071

detected, GeneScan analysis also provides a means for assessing the relative contribution of each transcript to the tumor levels. As the sensitivity of detection of fluorescently labeled amplification products by laser-based capillary electrophoresis is substantially superior to that of ethidium bromide-based detection, coupling real-time RT-PCR with capillary electrophoresis and GeneScan analysis allows easy identification of fusion transcript type even at very low tumor burden.

We have shown the utility of this assay in two separate patient groups. Initially, we randomly selected 26 samples of patients known to have *bcr-abl*-positive leukemias, 24 patients with chronic myelogenous leukemia and two with precursor B-cell acute lymphoblastic leukemia. As most of these patients were being treated and were at various stages of their clinical course, there was a wide spectrum of *bcr-abl* copy number, ranging from 16 162 to 0 copies detected. All six patients in whom no *bcr-abl* copies were detected had no morphologic or cytogenetic evidence of disease. We followed up on these results by assessing nine patients sequentially, at time of diagnosis, and then at regular intervals after onset of therapy at our institution. As expected, the RT-PCR assay detected relatively high levels at time of diagnosis and much lower levels subsequently, with the greatest drop in *bcr-abl* copy number occurring in the first 3 months after onset of therapy. These results illustrate that this modified RT-PCR method to assess *bcr-abl* is useful for *de novo* diagnosis and for monitoring response to therapy and minimal residual disease in patients with *bcr-abl*-positive leukemias.

Although relatively few studies are currently available regarding the significance of bcr-abl transcript type, some preliminary reports suggest that knowledge of transcript type may have clinical meaning or help us to further understand the pathogenesis of t(9;22)-positive leukemias. For example, Perego *et al*[28] reported that chronic myelogenous leukemia patients with b3a2 transcripts had higher platelet counts than those with b2a2 transcripts. In another study of chronic phase chronic myelogenous leukemia patients, Prejzner[29] suggested that patients with b3a2 transcripts had longer survival than those with b2a2 transcripts. Dual transcription of b2a2 and b3a2 transcripts may be linked to a polymorphism within the *bcr* locus.[30] Several investigators have recently reported detection of low levels of e1a2 transcripts, in addition to b2a2 or b3a2 transcripts, in most patients with chronic phase chronic myelogenous leukemia.[31–33] These e1a2 transcripts, thought to be derived from alternative splicing of b2a2 or b3a2 fusion RNA, may be clinically significant. In patients with acute lymphoblastic leukemia, Radich *et al*[34] have shown that detection of *bcr-abl* transcripts after allogeneic stem cell transplantation is highly predictive of eventual relapse in acute lymphoblastic leukemia patients with e1a2/p190, as compared with patients with b3a2/p210. Thus, identification of the type of fusion transcript, in addition to its utility for ruling out false positives and cross contamination, may

Table 5 Results of the modified *bcr-abl* real-time RT-PCR of specimens derived at initial diagnosis and at various intervals following therapy from eight patients with chronic myelogenous leukemia and a patient with acute lymphoblastic leukemia (# 8)

Patient #	*Time course*	*Transcript*	bcr-abl	abl	bcr-abl/abl × *100*
1	Initial	b3a2	17 516	44 200	39.629
	3-month FU	b3a2	10	21 135	0.047
	6-month FU	b3a2	5	39 161	0.013
	9-month FU	b3a2	7	91 164	0.008
	12-month FU	b3a2	0	21 460	0.000
2	Initial	b3a2	3800	37 500	10.133
	3-month FU	b2a2 and b3a2	8	230 000	0.003
	6-month FU	Negative	0	36 000	0.000
	12-month FU	Negative	0	200 722	0.000
3	Initial	b3a2	5900	14 000	42.143
	6-month FU	b3a2	13	98 900	0.013
	12-month FU	Negative	0	30 931	0.000
4	Initial	b3a2	832	37 400	2.225
	3-month FU	b3a2	144	69 000	0.209
	6-month FU	b3a2	0	27 343	0.000
	9-month FU	b3a2	1	28 666	0.003
	12-month FU	b3a2	6	24 223	0.025
	18-month FU	Negative	0	49 835	0.000
5	Initial	b3a2	760	14 700	5.170
	3-month FU	b3a2	429	16 400	2.616
	6-month FU	b3a2	578	30 298	1.908
	9-month FU	b3a2	2	31 306	0.006
	12-month Fu	b3a2	1	147 098	0.001
	18-month FU	Negative	0	69 501	0.000
6	Initial	b3a2	65 847	100 195	65.719
	9-month FU	b3a2	329	40 012	0.822
	12-month FU	b3a2	9	41 673	0.022
	18-month FU	b3a2	1	29 334	0.003
7	Initial	b2a2	5391	14 500	37.179
	3-month FU	b2a2	53	22 200	0.239
	6-month FU	b2a2	16	12 339	0.130
	9-month FU	b2a2	1	36 933	0.003
	12-month FU	b2a2	4	42 802	0.009
	18-month FU	b2a2	6	73 007	0.008
8	Initial	e1a2	9437	706 000	1.337
	3-month FU	Negative	0	94 200	0.000
	6-month FU	Negative	0	31 384	0.000
	9-month FU	Negative	0	64 217	0.000
9	Initial	b2a2 and b3a2	11 430	39 500	28.937
	6-month FU	b2a2 and b3a2	16 162	55 631	29.052
	18-month FU	b2a2 and b3a2	37 280	62 907	59.262

Fu, follow up

prove valuable for assessing the clinical relevance of various transcripts in patients with the chronic myelogenous leukemia and t(9;22)-positive acute lymphoblastic leukemia. Furthermore, identification of the type of fusion transcript in a leukemia patient prior to BM or stem cell transplant is essential for assessing minimal residual disease after transplantation.

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