Chronic myeloid leukemia patients with the e13a2 *BCR-ABL* fusion transcript have inferior responses to imatinib compared to patients with the e14a2 transcript

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ABSTRACT

Background

Chronic myeloid leukemia is characterized by a reciprocal translocation between chromosomes 9 and 22, creating the fusion gene *BCR-ABL*. The clinical significance of the type of *BCR-ABL* transcript in newly diagnosed patients in chronic phase treated with imatinib 400 mg from initial diagnosis remains unknown.

Design and Methods

We analyzed the clinical outcome of 78 newly diagnosed chronic phase patients, aged 16 or over, treated with imatinib 400 mg. Of these, 71 expressed either e13a2 or e14a2 transcripts. *BCR-ABL* transcripts were assayed by quantitative real-time polymerase chain reaction.

Results

After 12 months of treatment, 54% of the e14a2 patients had achieved a complete cytogenetic response, compared to 25% of the e13a2 patients (p=0.01). Kaplan-Meier analysis of the time to achieve complete cytogenetic response revealed that e14a2 patients had more rapid response rates, compared to e13a2 patients (p=0.006). e14a2 patients had a higher event-free survival rate in the first 12 months of treatment, although overall survival did not differ significantly between the patients with the two types of transcript. Human organic cation transporter protein 1 mRNA levels did not differ between the patients with the two types of transcript. The pre-treatment pCrKL/CrKL ratio (a surrogate marker of BCR-ABL tyrosine kinase activity) was higher in patients with e13a2 transcripts than in those with e14a2 (p=0.017).

Conclusions

Patients expressing the e14a2 transcript type have a higher rate and more rapid complete cytogenetic responses than e13a2-expressing patients, which may be due to higher BCR-ABL tyrosine kinase activity. Knowledge of the transcript type may yield additional prognostic information, although this requires testing on larger datasets.

Key words: chronic myeloid leukemia, BCR-ABL fusion transcript, imatinib.

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Introduction

Chronic myeloid leukemia (CML) is characterized by a reciprocal translocation between chromosomes 9 and 22, which creates the fusion gene *BCR-ABL*. The breakpoints of chromosome 22 cluster within a small (5.8 kb) region, spanning exons e12-16 (formally denoted as b1-5) known as the major breakpoint cluster region (M-BCR). Breakpoint locations almost always fall between either exons e13 and e14 or between e14 and e15. Although the breakpoints in the *ABL* gene are also variable, because of splicing events, the transcribed mRNA has either an e13a2 (b2a2) or an e14a2 (b3a2) junction. The e13a2 and e14a2 *BCR-ABL* transcripts differ in length by 75 bp (25 amino acids).[1] Both *BCR-ABL* mRNA molecules encode a 210 kDa constitutively active protein kinase which is central to the pathogenesis of the disease.[2]

Imatinib is a tyrosine kinase inhibitor which has become the treatment of choice for newly diagnosed patients in chronic phase CML, and a recent report suggests that modern drug treatment may produce superior results to allogeneic stem cell transplantation.[3] We have recently shown in a population-based study[4] that by 24 months 49% of patients will fail imatinib treatment; similar findings were recently reported in a single center study.[5] This suggests that the identification of prognostic markers predictive of treatment response may be useful in order to avoid delay in offering second-line treatment such as stem cell transplantation or second-generation tyrosine kinase inhibitors.

Previous studies prior to the introduction of imatinib did not, in general, identify an effect of *BCR-ABL* transcript type on clinical outcome.[6-12] In the imatinib era one small study of 22 patients in different phases of disease suggested that patients with the e13a2 *BCR-ABL* transcript may be more sensitive to imatinib treatment;[13] while a larger study indicated that patients with e14a2 have a better molecular response to imatinib.[14] The clinical significance of the type of *BCR-ABL* transcript in newly diagnosed chronic phase CML patients treated with imatinib remains uncertain. Here we present the results of a population-based study in a single contiguous geographical locality investigating the effects of *BCR-ABL* transcript type on clinical outcome in 78 newly diagnosed patients with chronic phase CML treated with imatinib 400 mg.

Design and Methods

Patients and collection of samples

In our area of the north-west of England, the adjacent coastal strip of North Wales and the Isle of Man (total population 2 million), all services for adults with hematologic cancers are located in 12 hospitals. The molecular diagnosis of CML and monitoring for *BCR-ABL* transcripts for all CML patients in this geographical area are carried out in a single center (Royal Liverpool University Hospital). We are, therefore, able to trace the clinical course of every CML patient in our area. Peripheral blood samples were routinely collected at 3-monthly intervals for molecular monitoring. Briefly RNA was isolated from total white blood cells, cDNA was prepared and *BCR-ABL* transcripts were measured by real-time quantitative polymerase chain reaction (PCR), using a LightCycler as previously decribed.[15]

All 78 patients aged 16 or over with chronic phase CML newly diagnosed between January 1st 2003 and October 31st 2007 and with a minimum of 12 months follow-up were included in this study. The 71 patients who presented with either e13a2 or e14a2 *BCR-ABL* transcripts are the subject of the main investigation; patients presenting with both e13a2 and e14a2 transcripts are discussed separately (n=3). Patients expressing rare transcript types were excluded from this study (n=4; one each with e1a2, e14a3, e13a3 and one patient who expressed both e14a2 and e1a2 transcripts). Patients were included in the assessment of *BCR-ABL* transcript type if they received imatinib 400 mg daily from original diagnosis (preceded only by up to 6 weeks of hydroxycarbamide).

Measurement of CrKL phosphorylation by fluorescence-activated cell sorting

Phosphorylation of the CT10 regulator of kinase-like adaptor protein (CrKL) was used as a measure of BCR-ABL tyrosine kinase activity.[16] Cells (~5×10^5) were resuspended in 500 µL of 2% paraformaldehyde (VWR, Lutterworth, UK) and fixed for 10 min at 37°C. Cells were then chilled on ice for 1 min and centrifuged at 770 g for 3 min. Next, 500 µL of 90% methanol (Fisher Scientific, Leicestershire, UK) were added to the cell pellet. The cells were vortexed and then incubated on ice for 30 min. Cells were then washed (throughout with 1 mL incubation buffer containing phosphate-buffered saline and 0.5% bovine serum albumin), and centrifuged at 770g for 3 min. Cells were resuspended in 25 µL of incubation buffer and left at room temperature for 10 min. pCrKL antibody (28 µg/mL; Cell Signaling Technology, Massachusetts, USA) or CrKL antibody (28 µg/mL; Santa Cruz Biotechnology, California, USA) was added and 28 µg/mL anti-normal-rabbit immunoglobulins (R&D Systems, Abingdon, UK) used as a control. Cells were vortexed and incubated at room temperature for 40 before being washed twice and resuspended in 100 µL of incubation buffer containing 10 µg/mL fluorescein-labeled goat anti-rabbit second antibody Alexa Fluor 488 (Invitrogen, Paisley, UK), incubated at room temperature in the dark for 30 min, then washed twice and analyzed using flow cytometry (FACScalibur; Becton Dickinson, Oxford, UK), with Cellquest Pro software (Becton Dickinson) for data analysis. The pCrKL/CrKL ratio of a sample was determined using the following equation:

$$\text{pCrKL/CrKL ratio} = \frac{\text{pCrKL} - \text{control}}{\text{CrKL} - \text{control}} \times 100$$

Human organic cation transporter protein 1 mRNA analysis

Levels of human organic cation transporter protein 1 (hOCT1, SLC22A1) were determined using pre-treat-

ment cDNA, as previously described, on all suitable samples expressing either e13a2 or e14a2 transcripts.[17]

Clinical response

Responses were defined conventionally:[18] complete hematologic response was defined as normalization of the blood count and resolution of splenomegaly and complete cytogenetic response (CCR) was defined as no Philadelphia chromosome-positive metaphases among at least 20 bone marrow metaphases. In some cases serial cytogenetic data were not available and the achievement of CCR was determined by a *BCR-ABL/ABL* transcript ratio of less than 1%, which we have previously shown to be strongly correlated with cytogenetically defined CCR;[15] we, therefore, use the term *CCR equivalence* (CCRe) to encompass these cases.[4]

Statistical analysis

Statistical analysis and comparisons were performed using the statistical program SPSS 16.0 (SPSS Inc. Chicago, USA). Fishers' test and Mann-Whitney tests were used.

Results

During the study 78 patients with newly diagnosed chronic phase CML were assessed, of whom four expressed a rare/variant transcript type and were, therefore, excluded from this analysis. Three cases co-expressed e13a2 and e14a2 and for the purpose of this study they are considered as a separate group, leaving 71 cases expressing either e13a2 or e14a2 *BCR-ABL* transcripts. Of these, 32 expressed the e13a2 type of *BCR-ABL* transcript type and 39 expressed the e14a2 type of transcript. Age, sex and Sokal score of all cases analyzed are presented in Table 1.

Outcome according to transcript type

At 12 months 25% of patients with the e13a2 transcript had achieved a CCRe compared to 54% of e14a2 patients (p=0.01, Table 2). At 18 and 24 months the e13a2 patients continued to have lower rates of CCRe compared to those of the e14a2 patients. Kaplan-Meier analysis of time to achieve CCRe revealed that the patients with the e14a2 transcript type had more rapid responses than did the e13a2 patients (Figure 1A), with this effect continuing throughout treatment (p=0.006).

Patients with the e14a2 transcript type demonstrated a non-significant trend toward a higher event-free survival rate in the first 12 months of treatment, although overall survival rates did not differ significantly between patients with the two types of the transcript (Figure 1B and C). This trend is consistent with the finding that more patients with e13a2 progressed in the first 12 months of treatment; imatinib treatment failed in eight patients with e13a2 (7 had disease progression and 1 was intolerant to therapy), while treatment failed in only three patients with the e14a2 transcript (2 had disease progression and 1 was intolerant to therapy). The Sokal score was not predictive of clinical outcome nor did it differ according to the type of transcript (Table 1). Of interest, five additional cases outside this study who presented with blast crisis CML during the period of the study all expressed the e13a2 transcript type.

Additionally three cases presented with both e13a2 and e14a2 *BCR-ABL* transcripts at diagnosis. Following 12 months of treatment two achieved a CCRe, although by 24 months one of these patients had subsequently lost the CCRe. The patient who failed to

Table 1. (A) Summary of the patients' characteristics.

Number of patients	71	32	39
Age (years)	50 (19-81)	48 (19-75)	51 (19-81)
Sex (male/female)	36/35	19/13	17/22
Sokal score			
High	21	9	12
Intermediate	14	5	9
Low	15	6	9
No data	21	12	9

Table 1. (B) CCRe rates for both e13a2 and e14a2 *BCR-ABL* transcript types at 12, 18 and 24 months.*

	12 months			18 months			24 months		
	Total patients	CCRe	% of total patients achieving CCRe	Total patients	CCRe	% of total patients achieving CCRe	Total patients	CCRe	% of total patients achieving CCRe
Transcript type									
e13a2	32	8	25.0	23	8	34.8	23	9	39.1
e14a2	39	21	53.8	32	18	56.3	26	15	57.7

**At 18 months nine cases with e13a2 and seven cases with e14a2 are excluded from analysis as not yet having received 18 months of Imatinib treatment. At 24 months six further cases with e14a2 are excluded for the same reason.*

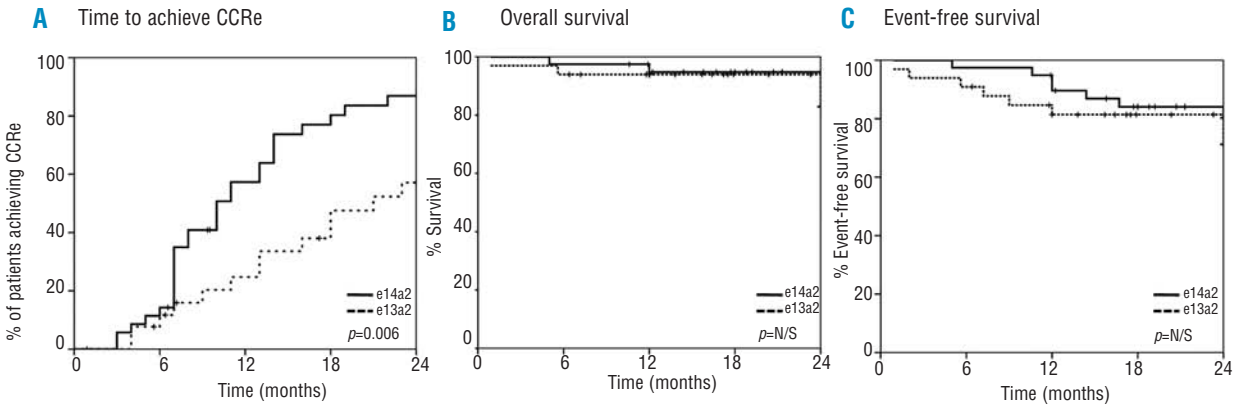

Figure 1. Kaplan Meier plots. (A) Kaplan Meier estimate of time to achieve CCRe for patients with the e13a2 and e14a2 *BCR-ABL* transcripts, demonstrating a slower response for e13a2 patients (p=0.006). (B) Overall survival stratified by *BCR-ABL* transcript type and (C) event-free survival; no statistically significant differences were observed (SPSS statistical package Mantel-Cox log-rank test).

achieve a CCRe following imatinib treatment was switched to an alternative tyrosine kinase inhibitor but failed to achieve a CCRe.

Correlation of transcript type with human organic cation transporter protein 1 and BCR-ABL kinase activity

Imatinib uptake into CML cells is dependent on hOCT1,[19] while the pCrKL/CrKL ratio is a surrogate marker for BCR-ABL tyrosine kinase activity.[16] In an attempt to establish why patients with the two different types of transcript respond differently to imatinib treatment we determined hOCT1 mRNA levels (n=51) and the pCrKL/CrKL ratio (n=28) in all patients for whom suitable material was available. No relationship was found between transcript type and hOCT1 mRNA (*data not shown*). However, samples from e13a2 patients had a higher pCrKL/CrKL ratio than those from e14a2 patients (p=0.017), demonstrating a higher tyrosine kinase activity (Figure 2).

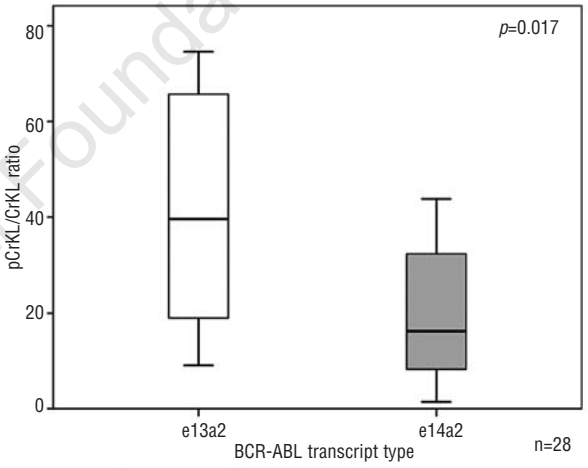

Figure 2. pCrKL/CrKL ratio for patients with the e13a2 and e14a2 transcripts pCrKL/CrKL ratio was measured in 28 newly diagnosed chronic CML patients prior to treatment as a surrogate marker for BCR-ABL tyrosine kinase activity, the pCrKL/CrKL ratio was higher in e13a2 patients than in e14a2 patients.

Discussion

In the pre-imatinib era, numerous studies addressed the significance of the *BCR-ABL* transcript type on CML outcome. Confusion can arise because some studies pooled e13a2 and e14a2 transcript types as 5' breakpoints; which were then compared to 3' breakpoints. These 3' breakpoints include e1a2 fusion transcripts that encodes a p190 protein, which is associated with acute lymphoblastic leukemia and has a poorer outcome than chronic phase CML. In general no difference in outcome was found in the pre-imatinib era between patients with chronic phase CML expressing e13a2 or e14a2 transcripts. However, surprisingly little information is available on the effects of these transcripts on response to imatinib treatment. In this study we focused on comparisons between outcomes in patients with e13a2 and e14a2 transcripts.

The e13a2 junction may be more prevalent in men,[20] although not necessarily in all ethnic groups, and has been associated with blast crisis of myeloid phenotype.[7] The e14a2 transcript has been correlated with a higher platelet count in both adults[8,10] and children.[21] Additionally it has been associated with a longer chronic phase and survival, possibly related to the less aggressive course of chronic phase in patients with this transcript type.[22] Furthermore, patients with the e14a2 transcript were found to have a higher level of 5'*ABL* deletions as an additional chromosomal abnormality, when compared to e13a2-expressing patients.[7] Other studies found no correlations with the above, or with any other clinical or hematologic parameters.[20,22-24]

Overall, data from the pre-imatinib era suggest that transcript type has no influence on clinical outcome.[6-12] However, to our knowledge, only two studies have addressed the effect of transcript type on the clinical outcome of patients treated with imatinib. One small study of 22 patients in Brazil with variable disease status found a significant difference in response to treatment at 6 months (p=0.0347), with e13a2-expressing patients responding better than those expressing e14a2.[13] A much larger study of patients in chronic phase treated with imatinib concluded that patients with e14a2 had a higher probability of achieving a major molecular response, and had a greater reduction in overall transcript levels in response to imatinib than did e13a2-expressing patients.[14]

Here we present additional data on all the patients in our contiguous geographical area. These data support the view that patients with the e14a2 transcript may respond better to imatinib 400 mg daily. Following 12 months of imatinib treatment e14a2 patients not only achieved significantly higher CCRe rates than e13a2 patients but also achieved their CCRe at a faster rate. This was not related to the patients' age, sex or Sokal score which takes into consideration standard hematologic parameters.[25] Additionally hOCT1 expression did not differ between e13a2 and e14a2 patients; thus, the degree of imatinib uptake between patients with the two types of transcript is similar and does not account for the observed differences in clinical responses.

The reason for the difference in response to imatinib therapy therefore appears to be unrelated to differences in imatinib transport between transcript types. It is, however, likely that the effect is due to differences in the drug target. Using the pCrKL/CrKL ratio as assessment of BCR-ABL tyrosine kinase activity,[16] it was found that tyrosine kinase activity is higher in e13a2 patients than in patients with e14a2. A fixed dose of imatinib may, therefore, suppress the lower kinase activity in e14a2 patients to a greater proportional extent than the higher kinase levels in e13a2 patients. This may explain the higher incidence of CCRe following treatment with imatinib in e14a2 patients.

In conclusion, patients with the e14a2 *BCR-ABL* transcript type have a higher response rate to imatinib when the CCRe rate is compared to patients who express the e13a2 transcript type. We suggest that this maybe due to e13a2 patients having a higher BCR-ABL tyrosine kinase activity. Knowledge of patient transcript type may yield clinically useful data, and should be included in future clinical trials of tyrosine kinase inhibitors.

Authorship and Disclosures

CML, RJH and REC designed the study and wrote the manuscript; CML, AG, AD and LW performed the laboratory work for this study; KK and SW were responsible for taking patients' samples and providing clinical information; REC was the principal investigator.

The authors reported no potential conflicts of interest.

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