Featured Article

Correlation of Major Cytogenetic Response with a Pharmacogenetic Marker in Chronic Myeloid Leukemia Patients Treated with Imatinib (STI571)

Marlene A. Dressman,[1] Rachel Malinowski,[1] Lee Anne McLean,[1] Insa Gathmann,[2] Renaud Capdeville,[2] Martee Hensley,[2] IRIS (International Randomized Study of Interferon-α *versus* STI571) Study Group, and Mihael H. Polymeropoulos[1]

[1]Clinical Pharmacogenetics, Novartis Pharmaceuticals Corp., Gaithersburg, Maryland, and [2]Oncology, Novartis Pharma AG, Basel, Switzerland

Abstract

Purpose: **Imatinib, an inhibitor of the Bcr-Abl tyrosine kinase, is indicated for the treatment of patients with Philadelphia chromosome-positive chronic myeloid leukemia. We examined genotypes from patients enrolled in the International Randomized Study of IFN-α *versus* STI571 in an attempt to identify factors that associate with cytogenetic response.**

Experimental Design: **Sixty-eight polymorphic loci in 26 genes were examined in a subset of 187 patients (imatinib-treated patients, $n = 113$; IFN + 1-β-D-arabinofuranosylcytosine-treated patients, $n = 74$). Correlations between genotype and major cytogenetic response (MCyR) were examined by Fisher's exact tests. Multivariate and survival analyses were also performed.**

Results: **A significant association between MCyR and the rs2290573 polymorphism mapped to 15q22.33 was observed in imatinib-treated patients ($P = 0.00037$, Bonferroni corrected $P = 0.025$). Individuals with a CC genotype at this locus had a MCyR rate of 52% compared with individuals with a CT or TT genotype that had a MCyR rate of 89% (odds ratio, 6.72; 95% confidence interval, 1.51–29.91). In a multivariate analysis, the rs2290573 polymorphism was significant, whereas Sokal score was not. Time to progression analysis illustrated a significant difference based on genotype for the rs2290573 polymorphism.**

Conclusions: **A significant association was identified between the genetic polymorphism rs2290573 and MCyR in imatinib-treated patients. This polymorphism is located in the intronic sequence of a putative gene with a tyrosine kinase domain. Multivariate analysis suggests that an individual's genotype for rs2290573 has more predictive value for MCyR than prognostic variables such as Sokal score. The clinical relevance of these results requires validation in future clinical trials.**

Introduction

Imatinib (Gleevec/Glivec, formerly STI571) is a selective, protein tyrosine kinase inhibitor of the BCR-ABL fusion protein. The BCR-ABL fusion protein, which results from a reciprocal translocation between (9,22)(q34;q11), is also known as the Philadelphia chromosome (Ph). Approximately 90% of chronic myelogenous leukemia cases are Ph+ (1–4). Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder with a well-defined clinical course of three phases of disease: chronic phase consisting of a median stabilization of 5–6 years, followed by accelerated phase with a median duration of 6–9 months, and blast phase with a median survival of 3–6 months. The only known cure for CML is stem cell transplantation (5). Alternatives to bone marrow transplantation include imatinib treatment, which has been approved for first-line therapy of chronic phase CML. The goal of this treatment option includes complete hematological remission and cytogenetic response.

Data from a Phase III randomized study comparing imatinib with IFN-α plus cytarabine [International Randomized Study of Interferon-α *versus* STI571 (IRIS); Ref. 6] demonstrated that in the first-line treatment of newly diagnosed CML patients, at 18 months after the last patient was recruited, 87.1% (95% confidence interval, 84.1–90.0) of patients treated with imatinib achieved major cytogenetic response (McyR). In contrast, for the IFN + 1-β-D-arabinofuranosylcytosine (Ara-C) treatment arm, 34.7% of patients achieved MCyR.

Polymorphisms in genes related to the leukemic process, drug target, absorption, distribution, metabolism, drug response, and excretion of imatinib may affect an individual's response to imatinib with regard to efficacy. The primary aim of this exploratory pharmacogenetic analysis was to identify genetic markers of cytogenetic response. Genotyping was performed for all 187 imatinib and IFN + Ara-C-treated patients from the United States (US) who consented to the pharmacogenetic protocol for 68 single nucleotide polymorphisms in 26 genes. The 26 candidate genes were chosen because of their involvement in absorption, distribution, metabolism, excretion, DNA repair, etiology of the disease, drug response, or drug mechanism of action.

Prediction score systems generated from microarray expression data have been reported that have the ability to differentiate between patients who responded to imatinib treatment

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Note: M. Dressman and R. Malinowski contributed equally to this work. Present address: M. Polymeropoulos, Vanda Pharmaceuticals, Rockville, Maryland 20850.
Requests for reprints: Marlene A. Dressman, Vanda Pharmaceuticals, 9620 Medical Center Drive, Rockville, Maryland 20850. Phone: (301) 294-9300; Fax: (301) 294-1900; E-mail: marlene.dressman@vandapharmaceuticals.com.

and those who did not respond (7, 8). Furthermore, polymorphisms in the *BCR-ABL* kinase domain have been identified in patients who experience a relapse of disease after initial response on imatinib (9). These discoveries, along with the identification of the rs2290573 polymorphism as a genetic marker of response reported here, could aid in elucidating which patients have the highest probability of achieving MCyRs to treatment with imatinib.

Materials and Methods

Patient Population. The IRIS study was composed of 1106 patients from 16 countries who were equally randomized to either imatinib or IFN + Ara-C treatment. A total of 428 patients were enrolled in the US. A total of 187 of these 428 patients consented to the pharmacogenetics protocol, which was approved by an institutional review board at each participating site, and were subsequently genotyped. One hundred and thirteen of these 187 patients were randomized to imatinib treatment, and 74 were randomized to IFN + Ara-C treatment.

To determine whether the subset of patients who were used for the pharmacogenetic studies were representative of the entire multinational trial population, we examined race, sex, and age in three populations. The first population was composed of the subset of imatinib- and IFN-treated patients from the IRIS trial that were genotyped, the second population was composed of patients enrolled in the clinical trial from the US, and the third population was composed of the entire clinical trial population. Analyses of continuous variables were performed by nonparametric ANOVAs, and all other demographics were analyzed by Fisher's exact tests.

Genotyping. Genotyping was performed for all 187 imatinib- and IFN-treated patients from the US who consented to the pharmacogenetics protocol for 68 single nucleotide polymorphisms in 26 genes (Table 1). Genotyping was performed on 20 ml of peripheral whole blood obtained on the first day of study before study drug administration. The DNA was extracted using the PUREGENE DNA Isolation Kit (D-50K; Gentra, Minneapolis, MN) according to the manufacturer's recommendations. Genotyping was performed on 60 ng of genomic DNA using the Invader assay (Third Wave Technologies Inc., Madison, WI) according to the manufacturer's recommendations (10).

Tests for Hardy-Weinberg equilibrium (HWE) by Fisher's exact tests were performed to verify quality control and to test the study population for deviations in the genotype distribution. HWE was analyzed in the subset of genotyped individuals and in a subset of controls. The Human Variation Collection (HD100CAU, HD100AA) from the Coriell Institute for Medical Research (Camden, NJ) was used for the control samples.

Genotype/Phenotype Correlations. Correlations between genotype and MCyR were examined with Fisher's exact tests. Patients with complete or partial (≤35% Ph+ cells) cytogenetic response were classified as major cytogenetic responders, whereas those with minor (>35–65% Ph+ cells), minimal (>65–95% Ph+ cells), or no response (>95–100% Ph+ cells) were classified as nonresponders. MCyR was considered as confirmed when two consecutive bone marrow assessments at least 4 weeks apart showed ≤35% Ph+ cells.

Table 1 List of 26 genes with polymorphic variations studied for correlation with major cytogenetic response

Gene symbol	Gene description
ABCB1	ATP-binding cassette, subfamily B (MDR/TAP), member 1
ACE	Angiotensin converting enzyme
ACE2	Angiotensin 1 converting enzyme (peptidyl-dipeptidase A) 2
APOA1	Apolipoprotein 1A
BCR	Breakpoint cluster region
CYP1A1	Cytochrome P450, subfamily 1 (aromatic compound-inducible), polypeptide 1
DKFZP434C131	Putative tyrosine kinase gene
IL1A	Interleukin 1 α
IL1B	Interleukin 1 β
ITGB2	Integrin β 2
MXI1	MAX interacting protein 1
MYH7	Myosin heavy chain 7
NPPA	Natriuretic peptide precursor A
NR1I2	Nuclear receptor subfamily 1, group 1, member 2
PDGFA	Platelet-derived growth factor α polypeptide
PDGFB	Platelet-derived growth factor β
PDGFRA	Platelet derived growth factor receptor, α polypeptide
PDGFRB	Platelet derived growth factor receptor, β polypeptide
RPS6KB2	Ribosomal protein S6 kinase, 70kD, polypeptide 2
RPS6KB3	Ribosomal protein S6 kinase, 70kD, polypeptide 3
SCGF	Stem cell growth factor; lymphocyte secreted C-type lectin
SCNN1B	Sodium channel, nonvoltage-gated 1, β
TAL1	T-cell acute lymphocytic leukaemia 1
TNF	Tumor necrosis factor (TNF superfamily, member 2)
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
TP53	Tumor protein p53 (Li-Fraumeni syndrome)

Considering the most conservative approach, only confirmed responses were accounted for in this exploratory pharmacogenetic analysis. Therefore, the MCyR rate reported here is lower than those figures published previously from the IRIS study (6).

To most accurately assess response, patients who crossed-over or discontinued from the study without assessment of cytogenetic response were excluded from genotype/phenotype correlations. Of 113 imatinib-treated patients who were genotyped, 3 crossed over to the IFN treatment group, whereas 14 discontinued from the study. Therefore, 96 imatinib-treated patients were included in the analysis of first-line treatment. A significant number of IFN-treated patients in this subset crossed over to the imatinib arm ($n = 23$). Additionally, 31 IFN-treated patients discontinued from the trial and were not included in the analysis. Therefore, 20 of 74 IFN-treated patients were included in the analysis of first-line treatment. To correct for multiple testing, a Bonferroni correction factor was applied. All statistics were carried out in the statistical program SAS Version 8.2 (Cary, NC).

Fisher's exact tests were performed to investigate whether

there was a significant difference in genotype distribution for the racial categories defined by the clinical trial (Caucasian, African American, Oriental, and other). Hasford and Sokal prognostic scores have been used to predict survival among IFN-treated patients and may also be used to select patients for treatment (11, 12). We investigated whether these prognostic scores were associated with MCyR in this genotyped subset. We also examined whether the prognostic scores were associated with the rs2290573 polymorphism in imatinib-treated patients. The Hasford and Sokal Score formulas are shown below (11, 12):

Hasford score = 0.6666 × age [0 when age < 50 years; 1 otherwise] + 0.042 × spleen size [cm below costal margin] + 0.0584 × blasts (%) + 0.0413 × eosinophils (%) + 0.2039 × basophils [0 when basophils < 3%; 1 otherwise] + 1.0956 × platelet count [0 when platelets < 1500 × 10^9/liter; 1 otherwise] × 1000 Sokal score = exp (0.011 × (age − 43.4) + 0.0345 × (spleen size − 7.51) + 0.188 × ((platelets/700)^2 − 0.563) + 0.0887 × (blasts in peripheral blood–2.1)

A large percentage of the patients in this trial had been treated with hydroxyurea before being treated with imatinib. This included 86.5% of the 96 imatinib-treated patients evaluated for first-line treatment in this pharmacogenetic analysis. Furthermore, 52.1% of these 96 patients received concurrent treatment with hydroxyurea during the first 6 months of treatment with imatinib. A Fisher's exact test between hydroxyurea usage (both previous and concurrent) and MCyR was performed to investigate any relationship between these two variables in this subset.

Logistic Regression. A logistic regression was performed in imatinib-treated patients for significant findings. Logistic regression was used to determine which variables are predictive of MCyR. Only those patients who had been genotyped and randomized to treatment with imatinib were included in this analysis. IFN-treated patients were not examined with a logistic regression model due to the lack of significance for all polymorphisms studied by Fisher's exact tests. MCyR was used as the dependent variable, and genotype, race, and Sokal score were used as classes in the model. Genotypes for the two polymorphisms that significantly associated with MCyR were incorporated into the multivariate analysis. These included the C-511T *IL1B* polymorphism, coded as CC and CT/TT, and the rs2290573 polymorphism, coded as CC and CT/TT.[3] Due to the low prevalence of individuals classified as African American, Oriental, and Other, we collapsed the racial groups into two categories, Caucasian and non-Caucasian. Patients with an unknown Sokal score (n = 43) were not included in the model. Five additional patients were removed from the analysis because they discontinued treatment without assessment of MCyR. Of the 113 imatinib-treated patients genotyped, 65 were included in the model. All covariates (genotype, race, and Sokal score) were included in the full model. As a first assessment, the covariates were examined as independent main effects only. As a further analysis, the model was altered in such a way that it allowed up to two-way covariate interactions.

Time to Progression (TTP). We then investigated significant findings by TTP. The product-limit method (Kaplan-Meier) was used to estimate the survival function directly from the continuous survival and failure times. TTP was defined as the time from randomization to either death, increase in WBC count, loss of complete hematological response, loss of McyR, or progression to accelerated phase or blast crisis (6). Only patients randomized to imatinib were included in the pharmacogenetic analysis of TTP. There were only 5 individuals of the 113 imatinib-treated patients who experienced an event of progression in the 12 months after patient recruitment. To increase statistical power, data from an additional 6 months of follow-up was used in the TTP analysis, with 11 events of progression in the genotyped population. The analysis was stratified by genotype for the rs2290573 polymorphism to determine whether individuals with a CC genotype have a significantly shorter TTP compared with individuals with CT/TT genotypes at this locus.

Results

Patient Population. We compared demographics including race, sex, and age between the subset of patients genotyped, the patients enrolled in the clinical trial from the US, and the entire clinical trial population. The genotyped population, which was collected at US centers only, was not statistically different from the trial population from the US with regard to sex, race, and age (data not shown). The genotyped population compared with the entire trial is similar for sex and age, but different with regard to race. The genotyped population has more blacks (11.76% compared with 4.7%), fewer Caucasians (80.21% compared with 89.87%), and more individuals in the Other category (5.88% *versus* 3.80%.) This discrepancy is not surprising, given the racial diversity of the US population.

Genotype/Phenotype Correlations. Analysis of 68 genetic polymorphisms in 26 genes identified a significant association between the rs2290573 polymorphism and MCyR in imatinib-treated patients (P = 0.00037, Bonferroni corrected P = 0.025; Table 2). The association was significant when the Fisher's exact test was repeated with an additional 6 months of follow-up data (P = 0.001; data not shown). The rs2290573 polymorphism lies within the intronic region of putative gene *DKFZP434C131*, on 15q22.33, and represents a C/T base tran-

Table 2 Genotype distribution of the rs2290573 polymorphism in imatinib-treated patients stratified by major cytogenetic response

Numbers in parentheses represent the number of individuals that would be expected assuming no genotype/phenotype association between rs2290573 and MCyR.[a]

Observed (expected)	CC	CT[b]	TT	Total
MCyR	12 (18)	47 (43)	18 (16)	77
No MCyR	11 (5)	6 (10)	2 (4)	19
Total	23	53	20	96

[a] MCyR, major cytogenetic response.
[b] *P*-values were calculated by Fisher's exact tests of CC genotype *versus* CT/TT genotype (P = 0.00037).

[3] The Single Nucleotide Polymorphism Consortium LTD. Single Nucleotide Polymorphisms for Biomedical Research [online] Available: http://snp.cshl.org/(Accessed 31 Oct. 2002).

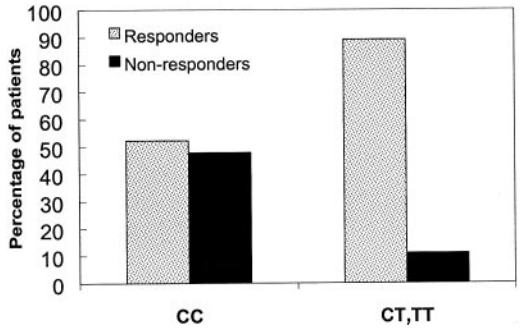

Fig. 1 Association between rs2290573 polymorphism and major cytogenetic response. Individuals with a CC genotype for the rs2290573 polymorphism are 6.72 times more likely not to achieve major cytogenetic response when treated with imatinib compared with individuals with a CT or TT genotype (95% confidence interval, 1.51–29.91). The *P*-value associated with this graph is 0.00037 (Bonferroni corrected $P = 0.025$).

sition.[4] The analysis demonstrated that imatinib-treated individuals with a CC genotype at this locus had a MCyR rate of 52%, whereas individuals with a CT or TT genotype had a MCyR rate of 89%, Fig. 1 (odds ratio, 6.72; 95% confidence interval, 1.51–29.91). The overall MCyR rate in this population is 80.2%. The rs2290573 polymorphism is in HWE in the population from the IRIS trial, which included both imatinib- and IFN-treated individuals.[5] The polymorphism was also in HWE in 92 Caucasian and 73 African-American controls. The allele frequency did not differ significantly between cases and controls when stratified by race.

An association between the rs2290573 polymorphism and MCyR was not observed in the small sample of IFN-treated individuals included in this analysis. Of the 20 IFN-treated patients analyzed, 8 achieved McyR, whereas 12 did not.

A preliminary analysis performed with 12 months of follow-up suggested a significant association between the C-511T *IL1B* polymorphism and MCyR in imatinib-treated patients ($P = 0.035$, Table 3). However, this was not confirmed with an additional 6 months of follow-up (data not shown). Excluding the rs2290573 and C-511T IL1B polymorphisms, none of the other 66 polymorphisms examined were significantly associated with MCyR in either the imatinib- or IFN + Ara-C–treated patients.

The distribution of CC:CT/TT individuals for the rs2290573 polymorphism is significantly different in imatinib-treated patients between Caucasians (10:67), African Americans (10:1), Orientals (2:1), and Others [1:4 (Table 4)]. Due to the racial difference in genotype distribution for rs2290573, we analyzed the correlation between genotype and MCyR stratifying by race classified as Caucasian and non-Caucasian. There was a significant association between the rs2290573 polymorphism and MCyR in the Caucasian group treated with imatinib at 12 months of follow-up ($P = 0.01$). In non-Caucasian patients treated with imatinib, a significant result was not observed between MCyR and the polymorphism. The trend in the Caucasian group that imatinib-treated individuals with a CC genotype have a greater chance of not responding than CT or TT individuals appears to be true in the non-Caucasian category also; however, the small sample size is not powered enough to detect significance (Table 4).

We observed a significant association between Sokal score and MCyR ($P < 0.01$) (data not shown). Sokal score did not significantly associate with the rs2290573 polymorphism. However, using three categories of Sokal score (low, intermediate and high risk) 31% of individuals with a CC genotype for the rs2290573 polymorphism had a high risk Sokal score, while only 8.7% of individuals with CT and TT genotypes had a high risk Sokal score. Hasford score does not significantly associate with the rs2290573 polymorphism.

Results of analyses indicated that there was no significant association between MCyR and previous or concurrent hydroxyurea usage in the 96 imatinib-treated patients studied for pharmacogenetic analysis of first-line treatment (data not shown).

Logistic Regression. The logistic regression model enabled us to predict MCyR in relation to several prognostic variables including genotype, race, and Sokal score. The full model indicated that the rs2290573 polymorphism is significant in the model at the $\alpha = 0.05$ level of significance. This result was confirmed in data from an additional 6 months of follow-up (data not shown). The other variables were not significant, and we therefore conclude that the genotype of an individual at this locus is a better predictor of MCyR in this population than race or Sokal score (Table 5).

Table 3 Genotype distribution of the C-511T *IL1B* polymorphism in imatinib-treated patients stratified by major cytogenetic response

Numbers in parentheses represent the number of individuals that would be expected if the polymorphisms were not associated with MCyR.[a]

Observed (expected)	CC	CT[b]	TT	Total
MCyR	33 (29)	30 (33)	14 (15)	77
No MCyR	3 (7)	11 (8)	5 (4)	19
Total	36	41	19	96

[a] MCyR, major cytogenetic response.
[b] *P*-value were calculated by Fisher's exact tests of CC genotype *versus* CT/TT genotype ($P = 0.035$).

Table 4 Genotype distribution of the rs2290573 polymorphism in imatinib-treated patients stratified by racial category and MCyR[a]

	CC		CT/TT	
	MCyR	No MCyR	MCyR	No MCyR
Caucasian	5	5	59	8
African American	5	5	1	0
Oriental	1	1	1	0
Other	1	0	4	0

[a] MCyR, major cytogenetic response.

[4] National Center for Biotechnology Information Website. Sequence data and genetic maps [online]. Available: http://www.ncbi.nlm.nih.gov/ (Accessed 31 Oct. 2002).
[5] NPGN Novartis Pharmacogenetics Network Database (NPGN). Available internally: http://www.cdma.dev.pharma.novartis.intra/pg/index.html (Accessed 25 Oct. 2002).

Table 5 Summary of logistic regression analysis of MCyR[a]

Effect	*P*	Odds ratio	95% Confidence interval
rs2290573 (CC)	0.012	6.72	(1.51–29.91)
C-511T *IL1B* (CC)[b]	0.258	0.40	(0.08–1.98)
Race (Caucasian)[c]	0.298	2.90	(0.39–21.52)
Sokal1 (intermediate risk)[d]	0.653	1.53	(0.24–9.87)
Sokal2 (high risk)[e]	0.055	0.15	(0.022–1.04)

[a] Model utilized major cytogenetic response (MCyR) as dependent variable and included imatinib-treated patients ($n = 65$).

[b] The C-511T *IL1B* polymorphism was coded as CC and CT/TT, and the rs229057 polymorphism was coded as CC and CT/TT.

[c] Due to the low prevalence of African American, Oriental, and Other individuals, we transformed the racial groups into two categories, Caucasian and Other. Patients with an unknown Sokal score were not included in the model.

[d] The Sokal1 classification compares patients with an intermediate risk to those with a low risk.

[e] The Sokal2 classification compares patients with a high risk to those with a low risk.

Time To Progression. Based on data collected up to 18 months after patient recruitment, 11 of the 113 imatinib-treated genotyped patients had disease progression. Six of 29 imatinib-treated patients (21%) with a CC genotype for the rs2290573 polymorphism experienced progression events. Five of 84 patients (6%) with a CT or TT genotype for the rs2290573 polymorphism experienced progression events (Table 6). A significant difference was observed between genotype and TTP according to the log-rank ($P = 0.018$) and Wilcoxon ($P = 0.023$) statistical tests (Fig. 2). This observation strengthens the association we reported between rs2290573 genotype and MCyR.

Discussion

Exploratory pharmacogenetic analysis was performed on a subset of patients from the IRIS trial to identify genetic markers of MCyR. We performed statistical tests to identify any correlation between polymorphisms in candidate genes and the presence or absence of MCyR in CML patients in chronic phase of disease treated with either imatinib or IFN + Ara-C. We discovered a significant association between the rs2290573 polymorphism mapped to chromosomal region 15q22.33 and the response classification of MCyR in imatinib-treated patients (Fig. 3). This association was also significant in data from an additional 6 months of follow-up. The genotype/phenotype association is remarkable because of the high McyR rate of CML patients in the IRIS trial. The 52% of responders with a CC genotype at the putative gene locus are a considerable contrast to the 87.1% of responders observed in the IRIS study (6). Multivariate analysis revealed that the rs2290573 genetic polymorphism is more predictive of MCyR than known clinical variables such as Sokal score in this subset. Furthermore, the group of imatinib-treated individuals with a CC genotype at this locus has a significantly greater proportion of patients who experienced a progression event compared with the group of imatinib-treated individuals with a CT or TT genotype based on survival analysis. Notably, 9.7% of individuals in the genotyped subset experienced an event of disease progression, whereas 7.9% of individuals in the entire IRIS Study experience a progression event (6). It is most likely that this does not represent a statistical difference and is simply due to the smaller sample size analyzed in this exploratory study.

A significant association between the rs2290573 polymorphism and MCyR was not observed in individuals treated with IFN. A number of IFN-treated patients crossed over to the imatinib arm ($n = 23$) or discontinued the study ($n = 34$) and were therefore not included in the assessment of first-line treatment. This could potentially bias our results; however we would not necessarily expect to see a similar result in IFN-treated patients because of the unique mechanism of action for each compound.

The association between rs2290573 and MCyR was a serendipitous discovery given the candidate gene approach. When the exploratory pharmacogenetics study was initiated, the genomic map at 15q22.33 was unstable. The polymorphism rs2290573 mapped near the CYP450 gene CYP1A1 (Fig. 3). Although there is no indication that CYP1A1 is involved in the metabolism of imatinib, the rs2290573 polymorphism was included as part of a panel of CYP450 single nucleotide polymorphisms. During the course of this study, the genomic map of 15q22.33 was updated, and build 33 of the human genome sequencing project maps rs2290573 to a putative gene, *DKFZP434C131*. It can be inferred from electronic annotation that *DKFZP434C131* has protein tyrosine kinase activity, an ATP binding domain, protein serine/threonine kinase activity, a protein amino acid phosphorylation domain, and transferase activity. *DKFZP434C131* appears to be widely expressed in a large number of normal and malignant tissue cDNA libraries recorded in the UniGene database including melanotic melanoma and a lymphoma cell line. Currently, it is unknown whether the rs2290573 polymorphism affects the expression level of *DKFZP434C131*. However, rs2290573 is located in an intron of *DKFZP434C131*, and therefore it is unlikely that this polymorphism has a significant impact on expression levels. It is more likely that it represents a marker of nearby genetic variation that is functionally responsible for the decreased response rates observed in individuals of a CC genotype at this locus. It is possible that there is a functional variant that is in linkage disequilibrium with the polymorphism associated with MCyR and is relevant to the tyrosine kinase inhibition activity of imatinib.

The objective of this exploratory pharmacogenetic study

Table 6 Time to progression analysis

	CC genotype $n = 29$ (%)	**CT, TT genotype** $n = 84$ (%)
Total no. of patients with events (progression)	6 (20.7)	5 (6.0)
Log-rank test/Wilcoxon test	$P < 0.03$	
Death (as primary reason for discontinuation)	0 (0)	0 (0)
Progression to AP or BC[a]	0 (0)	3 (3.6)
Loss of MCyR	4 (13.8)	0 (0)
Loss of CHR	1 (3.4)	2 (2.4)
Increase in WBC (approved by Study Management Committee)	1 (3.4)	0 (0)

[a] AP, accelerated phase; BC, blast crisis; CHR, complete hematologic response; MCyR, major cytogenetic response.

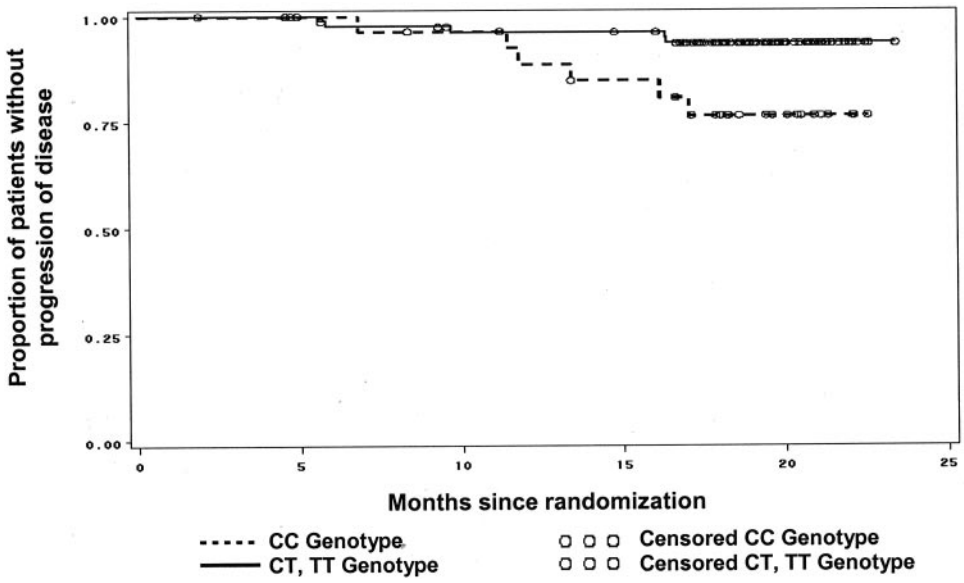

Fig. 2 Time to progression in imatinib-treated patients stratified by rs2290573 genotype. There were 6 of 29 imatinib-treated patients (21%) with the CC genotype for the rs2290573 polymorphism who experienced progression events. Five of 84 patients (6%) with the CT or TT genotype for the rs2290573 polymorphism experienced progression events. The time to progression was significantly different between CC and CT/TT genotype using log-rank test or Wilcoxon test ($P < 0.03$).

was to identify genetic markers that could be used to predict MCyR. The association between genetic marker rs2290573 and MCyR in imatinib-treated patients was discovered in DNA samples extracted from patient whole blood. There is a potential that the polymorphic site rs2290573 is mutated in the leukemic process. Whether the observed variation associated with MCyR is inherited or arose as part of the leukemic process does not impact on the significance of using this polymorphism as a biomarker for response. However, it could potentially identify different classes of leukemia if it is the result of somatic mutation. To address this issue, we tested for HWE in the patient population and in controls. The Hardy-Weinberg law states that allele frequencies do not change from generation to generation in a large population with random mating. Deviation from HWE in the patient population studied might suggest a technical error or involvement of the genetic polymorphism in the etiology of the disease. Both patients and controls were in HWE. Furthermore, the allele frequencies between patients and controls for this polymorphism were not significantly different when accounting for race. Therefore, it is highly unlikely that the observed genetic variation between responders and nonresponders is the result of a somatic mutation. While blast cells and a

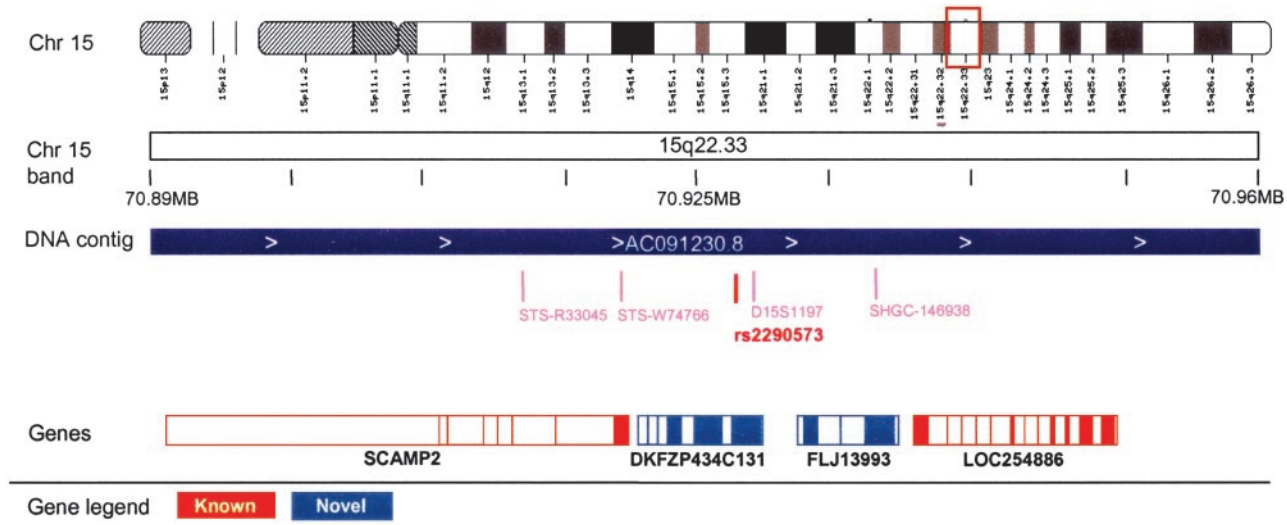

Fig. 3 Genetic map of 15q22.33 [Ensembl Genome Browser, sequence data and genetic maps (online), available at http://www.ensembl.org/ (accessed 31 Oct 2002); 6SNP Database Build 108, National Center for Biotechnology Information (online), available at http://www.ncbi.nlm.nih.gov/SNP/index.html. (accessed March 2002)]. The rs2290573 polymorphism is currently mapped to a putative gene, *DKFZP434C131*, with a tyrosine kinase domain. The genomic contig NT_010374.9, which was used to create the map of the region on chromosome 15, includes multiple breaks so that it is impossible at this time to determine the exact location of the polymorphism on the chromosome.

population of nonclonal cells from imatinib-treated patients are unavailable from this study, these cell types should be genotyped for rs2290573 in future studies to determine whether this locus is mutated in the leukemic process.

In conclusion, we have identified a significant association between a polymorphism in a putative gene with a tyrosine kinase domain and McyR in imatinib-treated CML patients. Future studies to (*a*) validate the association between the rs2290573 polymorphism and MCyR in a second population sufficiently powered to study the observed racial difference in genotype distribution, (*b*) determine whether the association is significant in individuals from different racial backgrounds, and (*c*) further characterize the putative gene *DKFZP434C131* with regard to CML etiology and response to imatinib would be valuable steps for evaluating the clinical relevance of this finding.

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Appendix

The following investigators participated in the IRIS Study: Australia, T. P. Hughes (Adelaide), K. Taylor (Brisbane), S. Durrant (Brisbane), A. Schwarer (Melbourne), D. Joske (Perth), J. Seymour (Melbourne), A. Grigg (Melbourne), D. Ma (Sydney), C. Arthur (Sydney), K. Bradstock (Sydney), and D. Joshua (Sydney); Austria, K. Lechner (Wien); Belgium, G. Verhoef (Leuven), A. Louwagie (Brugge), P. Martiat (Bruxelles), N. Straetmans (Bruxelles), and A. Bosly (Yvoir); Canada, J. Shepherd (Vancouver), C. Shustik (Montreal), J. Lipton (Toronto), S. Couben (Halifax), I. Walker (Hamilton), D-C. Roy (Montreal), M. Rubinger (Winnipeg), I. Bence-Bruckler (Ottawa), D. Stewart (Calgary), M. Kovacs (London), and A. R. Turner (Edmonton); Denmark, J. L. Nielsen (Aarhus), H. Birgens (Herlev), and O. Bjerrum (Copenhagen); France, F. Guilhot (Poitiers), J. Reiffers (Pessac), P. Rousselot (Paris), T. Facon (Lille), J-L. Harousseau (Nantes), M. Tulliez (Créteil), A. Guerci (Vandoeuvre-les-Nancy), D. Blaise (Marseille), F. Maloisel (Strasbourg), and M. Michallet (Lyon); Germany, A. Hochaus (Mannheim), T. Fischer (Mainz), D. Hossfeld (Hamburg), R. Mertelsmann (Freiburg), R. Andreesen (Regensburg), C. Nerl (München), M. Freund (Rostock), N. Gattermann (Düsseldorf), K. Hoeffken (Jena), G. Ehninger (Dresden), M. Deininger (Leipzig), O. Ottmann (Frankfurt), C. Peschel (München), S. Fruehauf (Heidelberg), A. Neubauer (Marburg), P. Le Coutre (Berlin), and W. Aulitzky (Stuttgart); Italy, M. Baccarani (Bologna), G. Saglio (Orbassano), R. Fanin (Udine), G. Rosti (Bologna), F. Mandelli (Rome), E. Morra (Milan), A. Carella (Genova), M. Lazzarino (Pavia), M. Petrini (Pisa), P. Rossi Ferrini (Florence), F. Nobile (Reggio Calabria), V. Liso (Bari), F. Ferrara (Naples), V. Rizzoli (Parma), G. Fioritoni (Pescara), and G. Martinelli (Milan); the Netherlands, J. Cornelissen (Rotterdam) and G. Ossenkoppele (Amsterdam); New Zealand, P. Browett (Auckland); Norway, T. Gedde-Dahl (Oslo), J-M. Tangen (Oslo), and I. Dahl (Tromso); Spain, F. Cervantes (Barcelona), J. Odriozola (Madrid), J. C. Hernández Boluda (Valencia), J. L. Steegman (Madrid), C. Cañizo (Salamanca), A. Sureda (Barcelona), J. Diaz (Madrid), A. Granena (Llobregat), and M. N. Fernández (Madrid); Sweden, B. Simonsson (Uppsala), L. Stenke (Stockholm), C. Paul (Stockholm), M. Bjoreman (Orebro), C. Malm (Linköping), H. Wadenvik (Göteborg), P-G. Nilsson (Lund), and I. Turesson (Malmo); Switzerland, A. Gratwohl (Basel), U. Hess (Sankt Gallen), and M. Solenthaler (Bern); United Kingdom, S. G. O'Brien (Newcastle), J. M. Goldman (London), N. Russel (Nottingham), G. Mufti (London), J. Cavenagh (London), R. E. Clark (Liverpool), A. R. Green (Cambridge), T. L. Holyoake (Glasgow), G. S. Lucas (Manchester), G. Smith (Leeds), D. W. Milligan (Birmingham), S. J. Rule (Plymouth), and A. K. Burnett (Cardiff); US, B. J. Druker (Portland), R. A. Larson (Chicago), R. Moroose (Orlando), M. Wetzler (Buffalo), J. Bearden (Spartanburg), R. Brown (St. Louis), M. Lobell (Tucson), S. Cataland (Columbus), I. Rabinowitz (Albuquerque), B. Meisenberg (Baltimore), J. Gabrilove (New York), K. Thompson (Montgomery), S. Graziano (Syracuse), P. Emanuel (Birmingham), H. Gross (Dayton), P. Cobb (Billings), R. Bhatia (Duarte), S. Dakhil (Wichita), D. Irwin (Berkeley), B. Issell (Honolulu), S. Pavletic (Omaha), P. Kuebler (Columbus), E. Layhe (East Lansing), P. Butera (Providence), J. Glass (Shreveport), J. Moore (Durham), B. Grant (Burlington), H. Niell (Memphis), R. Herzig (Louisville), H. Burris (Nashville), H. Kantarjian (Houston), B. Peterson (Minneapolis), B. Powell (Winston-Salem), M. Kalaycio (Cleveland), D. Stirewalt (Seattle), W. Samlowski (Salt Lake City), E. Berman (New York), S. Limentani (Charlotte), T. Seay (Atlanta), T. Shea (Chapel Hill), L. Akard (Beech Grove), G. Smith (Farmington), P. Becker (Worcester), S. DeVine (Chicago), R. Hart (Milwaukee), R. Veith (New Orleans), J. Wade (Decatur), M. Brunvand (Denver), R. Silver (New York), L. Kalman (Miami), D. Strickland (Memphis), M. Shurafa (Detroit), A. Bashey (La Jolla), R. Shadduck (Pittsburgh), S. Cooper (Nashville), H. Safah (Orleans), M. Rubenstein (Campbell), R. Collins (Dallas), A. Keller (Tulsa), R. Stone (Boston), M. Tallman (Chicago), D. Stevens (Louisville), A. Pecora (Hackensack), M. Agha (Pittsburgh), and H. Holmes (Dallas).

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