Early prediction of success or failure of treatment with second-generation tyrosine kinase inhibitors in patients with chronic myeloid leukemia

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ABSTRACT

Background

Second-generation tyrosine kinase inhibitors induce cytogenetic responses in approximately 50% of patients with chronic myeloid leukemia in chronic phase in whom imatinib treatment has failed. However, it has not yet been established which of the patients in whom imatinib treatment fails are likely to benefit from therapy with second-generation tyrosine kinase inhibitors.

Design and Methods

We analyzed a cohort of 80 patients with chronic myeloid leukemia who were resistant to imatinib and who were treated with dasatinib or nilotinib while still in first chronic phase. We devised a scoring system to predict the probability of these patients achieving complete cytogenetic response when treated with second-generation tyrosine kinase inhibitors.

Results

The system was based on three factors: cytogenetic response to imatinib, Sokal score and recurrent neutropenia during imatinib treatment. We validated the score in an independent group of 28 Scottish patients. We also studied the relationship between cytogenetic responses at 3, 6 and 12 months and subsequent outcome. We classified the 80 patients into three categories, those with *good risk* (n=24), *intermediate risk* (n=27) and *poor risk* (n=29) with 2.5-year cumulative incidences of complete cytogenetic response of 100%, 52.2% and 13.8%, respectively ($P<0.0001$). Moreover, patients who had less than 95% Philadelphia chromosome-positive metaphases at 3 months, those with 35% or less Philadelphia chromosome-positive metaphases at 6 months and patients in complete cytogenetic response at 12 months all had significantly better outcomes than patients with lesser degrees of cytogenetic response.

Conclusions

Factors measurable before starting treatment can accurately predict response to second-generation tyrosine kinase inhibitors. Cytogenetic responses at 3, 6 and 12 months may influence the decision to continue treatment with second-generation tyrosine kinase inhibitors.

Key words: early prediction, tyrosine kinase inhibitors, chronic myeloid leukemia.

Citation: Milojkovic D, Nicholson E, Apperley JF, Holyoake TL, Shepherd P, Drummond MW, Szydlo R, Bua M, Foroni L, Reid A, Khorashad JS, de Lavallade H, Rezvani K, Paliompeis C, Goldman JM, and Marin D. Early prediction of success or failure of treatment with second-generation tyrosine kinase inhibitors in patients with chronic myeloid leukemia. Haematologica. 2010; 95:224-231. doi:10.3324/haematol.2009.012781

Funding: we are grateful for support from the UK NIHR Biomedical Research Centre Funding Scheme.

Manuscript received on June 16, 2009. Revised version arrived on July 7, 2009. Manuscript accepted on August 5, 2009.

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Introduction

First-line tyrosine kinase inhibitor (TKI) therapy with imatinib has resulted in outstanding clinical responses in patients with chronic myeloid leukemia (CML) in chronic phase;[1] however, despite the excellent results, approximately one third of imatinib-treated patients discontinue therapy due to an inadequate response or toxicity.[2] Second-generation TKI (2G-TKI), such dasatinib and nilotinib, can be effective after treatment with imatinib has failed,[3-8] but in practice fewer than 50% of patients actually obtain durable complete cytogenetic responses, although patients who do achieve good cytogenetic responses are most likely to obtain long-term benefit.[9] The association between cytogenetic response and long-term survival has been clearly demonstrated for patients treated with interferon or imatinib[1,10] and the achievement of a complete cytogenetic response is now generally accepted as a surrogate marker for survival. We, therefore, believe that it is important to predict the response to 2G TKI or to assess the likelihood of response in a given patient as early as possible after starting such treatment, because this will define the patients' risk.

We devised a scoring system for patients deemed resistant to imatinib which allows us to identify those patients who will benefit most from 2G TKI. We did not consider patients who stopped imatinib on account of non-hematologic toxicity, as such patients likely represent a biologically different group with a better prognosis. We validated the scoring system in an independent cohort of patients. We also explored the relationships between molecular and cytogenetic responses at 3, 6 and 12 months after starting treatment with a 2G TKI and progression-free survival and overall survival, providing further information about the value of 2G TKI therapy.

Design and Methods

Patients

Between March 2005 and Jan 2008, 80 consecutive patients with CML in chronic phase resistant to imatinib were treated with dasatinib (n=67) or nilotinib (n=13) at the Hammersmith Hospital in various phase II clinical studies. Written informed consent was obtained from all patients before enrollment. The characteristics of the patients were typical of those with imatinib-treated *late* chronic phase CML (Table 1). The median follow up for the surviving patients after starting 2G TKI was 28.3 months (range, 6.5-42); 97% of the patients were followed for at least 1 year. Dasatinib and nilotinib were administered as described by others.[6,7,11,12] Briefly, nilotinib was started at a dose of 400 mg every 12 h and dasatinib at a dose of either 70 mg every 12 h (n=23) or 100 mg once daily (n=44). Doses were adjusted according to tolerance.[6,7]

Chronic phase and complete hematologic response were defined by conventional criteria.[13,14] Bone marrow morphology and cytogenetics were assessed at diagnosis and then every 3 months. A complete cytogenetic response was defined by the failure to detect any Philadelphia chromosome (Ph)-positive metaphases in two consecutive bone marrow examinations. A partial cytogenetic response was defined as a decrease in the proportion of Ph-positive metaphases to between 1 and 35%, a major cytogenetic response was defined by combining the number of complete and partial cytogenetic responses, and a minor cytogenetic response was defined as a decrease in the proportion of Ph-positive metaphases to between 35 and 95%.

Detection of BCR-ABL transcripts and BCR-ABL kinase domain mutations

BCR-ABL transcripts were measured in the blood at 6 to 12 week intervals using real time quantitative reverse transcription polymerase chain reaction, as described previously.[2,15-18] Major molecular response was defined as a 3-log reduction in transcript levels from a standardized baseline[19] based on two consecutive molecular measurements, and complete molecular response as two consecutive samples with no detectable *BCR-ABL* transcripts, providing that the *ABL* control was equal to or greater than 10^4 copies. Samples obtained for the polymerase chain reaction were also analyzed for kinase domain mutations on a routine basis every 6 months using direct sequencing[20] and more often if resistance to imatinib was suspected.[2,21] Once a mutation was detected, earlier samples were analyzed to determine the time at which the mutation first became detectable.[2,21]

Statistical methods

Probabilities of overall, progression-free and event-free survival were calculated using the Kaplan-Meier method. Progression-free survival was defined as survival without evidence of accelerated or blastic phase disease.[13] In the evaluation of event-free survival, events were death from any cause, loss of a major or complete cytogenetic response, progression from chronic phase and loss of a complete hematologic response.

The probabilities of cytogenetic response and cytogenetic relapse were calculated using the cumulative incidence procedure, in which cytogenetic response or relapse represented the events of interest and death and disease progression were competing events. Univariate analyses were carried out using the log-rank test to identify prognostic factors for survival, progression-free survival, event-free survival and cytogenetic relapse. Variables found to be statistically significant at the P less than 0.20 level were entered into a proportional hazards regression analysis; a forward stepping procedure was employed to find the best model. The influence of kinase domain mutations and clonal evolution on the different outcomes was studied in a time-dependent Cox model. The proportional hazards assumption was confirmed by adding a time-dependent covariate for each covariate. Tests for interactions were carried out but none was found to have statistical significance. P values were two-sided and 95% confidence intervals (CI) were computed. As reported previously by others[9] we found no significant difference between dasatinib and nilotinib for any of the outcomes studied, which allowed us to consider the patients treated with these two drugs as a single cohort.

Calculation of a scoring system to predict cytogenetic response

The scoring system was calculated employing the methodology used previously by others to classify lymphoma.[22] Briefly we performed a multivariate analysis to identify independent factors that predict the likelihood of a given patient achieving a complete cytogenetic response and found four pre-therapy variables that were independently significant. One of these was the interval between the diagnosis of failure of imatinib treatment and the start of therapy with a 2G TKI. We did, however, think that this variable could be difficult to define in many centers and its inclusion might limit

the applicability of the scoring system (*see below*). We, therefore, performed a second multivariate analysis excluding this variable. In order to generate a scoring system we then ascribed a numerical value to the three factors resulting from the second analysis. A precise numerical value for each variable was a rounded number proportional to the inverse of the relative risk (RR) for achieving complete cytogenetic response for patients for that particular variable. A given patient's total score consisted of the sum of the numerical values derived from his or her status in relation to each of the three variables. Risk groups were defined by comparing the relative risk of response in patients with each possible number of points and combining categories with similar relative risks (e.g., 0 with 1). Patients were then assigned to one of three risk groups on the basis of their total score.[22] The 'good risk' group consisted of patients with scores less than 1.5, the 'intermediate risk' group was formed of patients with scores between 1.5 and 2.5 and the 'poor risk' group consisted of patients with scores greater than 2.5.

Results

Responses to second-generation tyrosine kinase inhibitors

With a median follow up of 28.3 months, 77 patients (96.3%) achieved or maintained a complete hematologic resonse, 46 (57.5%) achieved a major cytogenetic response, 42 (52.5%) achieved a complete cytogenetic response, 26 (32.5%) achieved a major molecular response and 2 (2.5%) achieved a complete molecular response. The 2.5-year cumulative incidences of major cytogenetic response, complete cytogenetic response and major molecular response were 57.5%, 52.6% and 27%, respectively (Figure 1).

We performed univariate and multivariate analyses in order to identify pre-therapy factors that predicted a complete cytogenetic response (Table 1). In univariate analysis the factors found to have a significant influence on the probability of achieving complete cytogenetic response were: the time between detecting failure of imatinib treatment (as defined by European LeukemiaNet criteria)[23] and starting therapy with a 2G TKI, the Sokal risk group (defined at diagnosis), the best level of cytogenetic response achieved during imatinib therapy, the presence of additional cytogenetic abnormalities in Ph-positive clones, the acquisition of hematologic resistance to imatinib, recurrent episodes of grade III-IV neutropenia during imatinib therapy that required dose reduction below 400 mg/day despite hematopoietic growth factor support[14] and the percentage of Ph-positive metaphases at the start of 2G TKI therapy.

The presence of kinase domain mutations prior to 2G TKI treatment did not affect the probability of achieving complete cytogenetic response (50% in patients with such mutations *versus* 46.5% in those without mutations, P=0.69). When the mutations were classified according to their degree of resistance to the chosen 2G TKI (based on *in vitro* studies),[24,25] we found that none of the four patients with mutations with an intermediate or high level of resistance achieved a complete cytogenetic response.

The multivariate analysis identified four pre-2G-TKI independent predictive factors for complete cytogenetic response, namely low Sokal risk score at diagnosis (RR=1.6, CI 1.1-2.4, P=0.01), the best cytogenetic response obtained on imatinib (0% Ph-positive, RR=1; 1-94% Ph-positive, RR=0.3, CI 0.2-0.54; more than 95% Ph-positive, RR=0.06, CI 0.02-0.17, P<0.0001), the occurrence of neutropenia at any time during imatinib therapy that required imatinib dose reduction below 400 mg/day despite

Table 1. Patients' characteristics at the time of starting second-generation-TKI treatment and 2.5-year probabilities of complete cytogenetic response (CCyR), event-free survival (EFS), progression-free survival (PFS) and overall survival (OS).

Variable	n	CuI* of CCyR (%)	EFS (%)	PFS (%)	Survival (%)
Age		P=0.67	P=0.81	P=0.63	P=0.70
>50 years	54	54.2	80.7	87.6	88.5
≤50 years	26	51.9	76.7	90.4	91.2
Sex,		P=0.64	P=0.20	P=0.50	P=0.34
Male	37	51.5	70.6	88.5	90.0
Female	43	53.5	85.8	90.5	92.1
Sokal risk group		P=0.02	P=0.03	P=0.17	P=0.15
Low	15	73.3	100	100	100
High + intermediate	65	47.8	73.0	86.8	87.3
Status at the onset of imatinib therapy		P=0.13	P=0.7	P=0.08	P=0.17
Newly diagnosed CP	48	47.9	78.8	93.4	95.4
Late CP	32	59.6	78.1	80.1	84.3
Additional cytogenetic abnormalities		P=0.01	P=0.08	P=0.47	P=0.55
No	58	62.2	82.5	91.3	91.5
Yes	22	27.3	67.5	84.8	86.4
Percentage of Ph-pos at start of second-generation-TKI,		P<0.0001	P=0.01	P=0.16	P=0.25
<95%	29	87.1	96.5	96.5	96.5
≥95%	51	33.3	69.2	86.1	86.9
Time from imatinib failure to second-generation-TKI		P<0.0001	P=0.04	P=0.05	P=0.15
≤6 months	19	82.3	87.3	93.6	93.6
> 6 months	61	45.1	63.0	79.8	82.9
Best cytogenetic response on imatinib **		P<0.0001	P=0.09	P=0.12	P=0.25
0% Ph-pos	25	84.0	93.2	95.2	96.1
1-94% Ph-pos	26	63.0	86.7	91.0	95.0
≥95% Ph-pos	29	17.2	74.6	80.4	80.4
Hematologic resistance to imatinib		P=0.002	P=0.008	P=0.6	P=0.8
Yes	27	29.6	58.5	88.3	89.2
No	53	64.3	88.5	90.4	91.0
Maximal dose of imatinib		P=0.8	P=0.64	P=0.73	P=0.57
400 mg/day	20	60.6	84.7	89.7	94.7
600 mg/day	31	51.6	72.1	86.4	87.5
800 mg/day	29	48.3	81.4	93.1	93.1
Kinase domain mutation		P=0.69	P=0.62	P=0.85	P=0.64
No	60	53.5	80.1	90.1	90.1
Yes	20	50.0	74.0	87.8	88.9
Recurrent neutropenia during imatinib treatment***		P=0.008	P=0.24	P=0.45	P=0.83
No	59	61.0	81.3	91.4	91.4
Yes	21	28.9	70.3	84.8	88.2

Cumulative incidence. **The P values for the differences between 0% and 1-94% Ph-pos and 1-94% Ph-pos and ≥95% Ph-pos were 0.008 and 0.0006, respectively. * Defined as recurrent episodes of grade III-IV neutropenia during imatinib therapy that required dose reduction below 400 mg/day in spite of growth factor support.*

growth factor support (RR=0.16, CI 0.64-0.42, *P*<0.0001) and the time in months from detection of imatinib failure to start of second 2G-TKI (>6 months RR=0.31, CI 0.1-0.57 *P*=0.001).

Scoring system to predict cytogenetic response

The score was calculated by allocating points (derived from the RR as described above) to each of the three variables as follows: (i) best cytogenetic response on imatinib: complete cytogenetic response, 0 points; 1-94% Ph-positive metaphases, 1 point; 95% or more Ph-positive metaphases, 3 points; (ii) Sokal risk group: low, 0 points; intermediate or high, 0.5 points; and (iii) neutropenia: no neutropenia, 0 points; recurrent episodes of grade III-IV neutropenia during imatinib therapy that required dose reduction,[14] 1 point. We decided not to consider the time from recognition of imatinib resistance to start of 2G TKI therapy in the scoring system because an accurate value is only available for patients who had marrow metaphase cytogenetics performed at the specified intervals after starting imatinib. We then divided the patients into three groups (Figure 2): the *good risk* group (n=24) consisted of patients with scores less than 1.5, the *intermediate risk* group (n=27) was formed of patients with scores between 1.5 and 2.5 and the *poor risk* group (n=29) consisted of the patients with scores greater than 2.5. At 2.5 years the cumulative incidences of complete cytogenetic response in these three groups were 100%, 52.2% and 13.8%, respectively (*P*<0.0001, Figure 2).

Validation of the prognostic score

The score was applied to an independent sample of 28 patients treated with a 2G TKI (22 with dasatinib and 6 with nilotinib) after failure of imatinib therapy. These patients were recruited in Glasgow and associated Scottish centers and their features were typical of patients in late chronic phase CML (*data not shown*). Of these 28 patients, 8 were classified as good risk, 8 as intermediate risk and 12 as poor risk. The 2.5-year cumulative incidences of complete cytogenetic response were 100%, 62.5% and 16.7% (*P*<0.0001), respectively. The *P* values for the differences between good and intermediate risk groups and between intermediate and poor risk groups were 0.03 and 0.02, respectively.

Probability of complete cytogenetic response according to cytogenetic response at 3 and 6 months

Of the 79 patients still in chronic phase at 3 months, 21 had achieved a complete cytogenetic response, 4 a partial cytogenetic response, 23 a minor cytogenetic resposne and 31 had no cytogenetic response. Patients who had achieved at least a minor cytogenetic response at 3 months had a significantly higher probability of achieving a complete cytogenetic response than the patients who had failed to achieve any degree of cytogenetic response (79.3% *versus* 0%, *P*<0.0001, Figure 3A). At 6 months 32 patients were in complete cytogenetic response, 8 in partial cytogenetic response, 6 in minor cytogenetic response and 32 had no cytogenetic response (one patient pro-

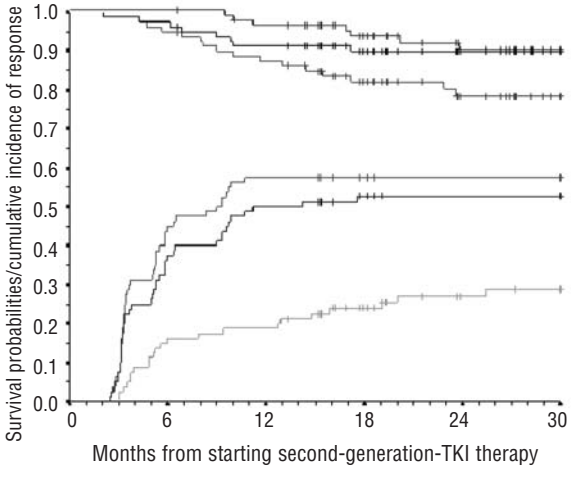

Figure 1. Event-free, progression-free and overall survival and cumulative incidences of major and complete cytogenetic responses and major molecular response in the Hammersmith population of patients (see text). For outcome (upper three lines): the top line indicates overall survival, the middle line indicates progression-free survival, the bottom line indicates event-free survival. For clinical response (lower three lines): the top line indicates cumulative incidence of major cytogenetic response, the middle line indicates cumulative incidence of complete cytogenetic response and the bottom line indicates cumulative incidence of major molecular response. Vertical lines indicate censored patients.

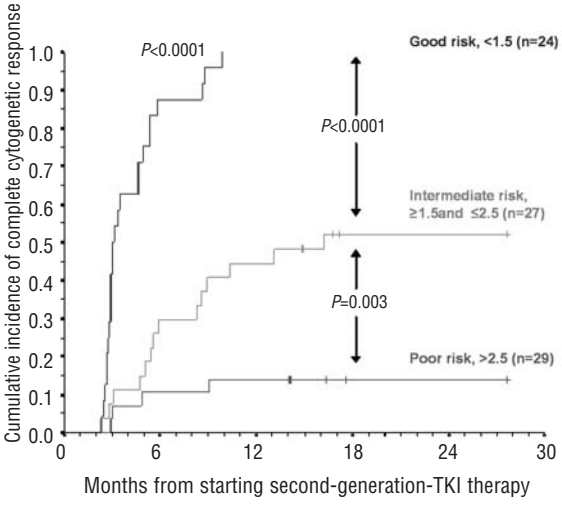

Figure 2. Hammersmith 3-criteria score for predicting cytogenetic responses to 2G-TKI therapy. The score can be calculated by allocating points to each of the three variables, as described in the text. Patients with a total score of <1.5 constitute the good risk group, those with a total score between 1.5 and 2.5 form the intermediate risk group, and those with a total score > 2.5 constitute the poor risk group. The 2.5-year cumulative incidences of complete cytogenetic response were 100%, 52.2% and 13.8%, respectively (*P*<0.0001). The score was validated with an independent sample of patients (see text). Vertical lines indicate censored patients.

gressed and two lost their cytogenetic response). For the patients who had achieved partial, minor or no cytogenetic response at 6 months the probabilities of achieving complete cytogenetic response during subsequent follow-up were 85.7%, 50% and 0% (P<0.0001), respectively (Figure 3B).

Event-free, progression-free and overall survival

Figure 1 shows the probabilities of event-free survival, progression-free survival and overall survival. Eleven of the 16 patients who had an 'event' and seven of the eight patients who progressed to advanced phase did so within the first year. Table 1 shows the 2.5-year probabilities of event-free, progression-free and overall survival according to variables defined at diagnosis. Patients belonging to the poor risk group according to our scoring system (see above) had worse 2.5-year event-free, progression-free and overall survival probabilities than patients belonging to the good risk group, namely 77.6% *versus* 100% (P=0.02) and 89.9% *versus* 100% (P=0.02), respectively (Figure 4). The event-free, progression-free and overall survival probabilities for patients in the intermediate risk group were 72.1%, 89.3% and 90.7%, respectively. These values are clearly better than those for the poor risk group and worse than those for the good risk group but the differences were not significant in all the cases (*data not shown*).

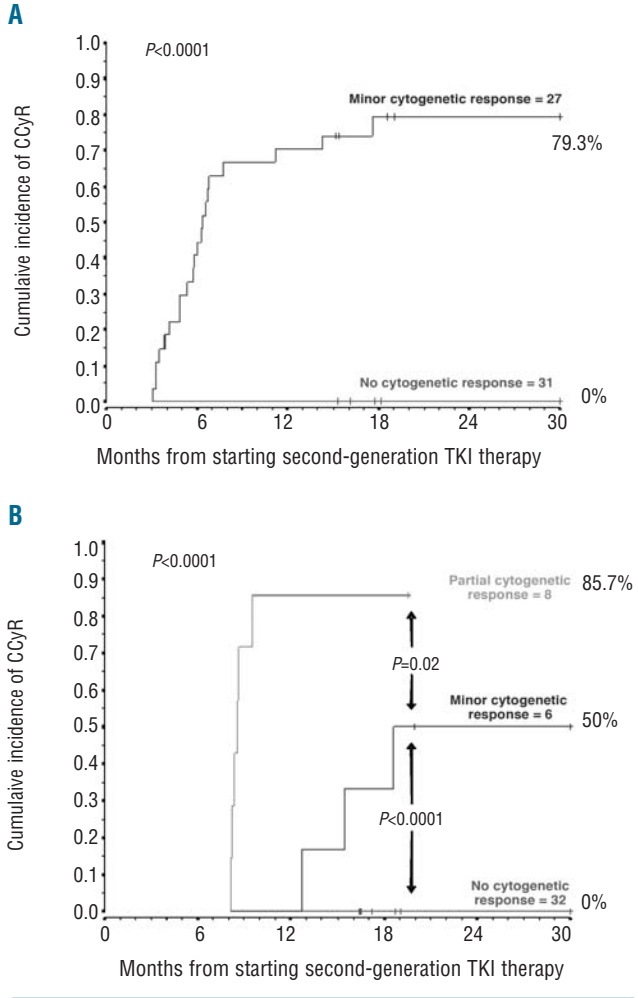

Figure 3. Cumulative incidence of complete cytogenetic response (CCyR) according to cytogenetic response at (A) 3 months, and (B) 6 months (see text). Panel A shows the cumulative incidence of CCyR according to the cytogenetic response at 3 months for the 58 patients who were not in CCyR at 3 months. Patients who were at least in minor cytogenetic response (MiCyR) at 3 months had a significantly higher probability of achieving CCyR than patients who had no cytogenetic response (79.3% *vs.* 0% P<0.0001). We found no difference in the probability of achieving CCyR between patients who were in partial cytogenetic response (PCyR) and those in MiCyR at 3 months (75% *vs.* 80.4%, P=0.7). Panel B shows the cumulative incidence of CCyR according to the cytogenetic response at 6 months for the 46 patients who were not already in CCyR at 6 months. The probabilities of achieving CCyR during the follow-up according to their cytogenetic response at 6 months were 85.7%, 50% and 0% (P<0.0001) for the patients who had achieved PCyR, MiCyR or no response respectively. The P values for the differences between partial and MiCyR and between MiCyR and no response were P=0.02 and P<0.0001 respectively. Vertical lines indicate censored patients.

Effects of response on outcome

At 3 months 79 patients were still in chronic phase. The 48 patients who had achieved at least a minor cytogenetic response had better event-free, progression-free and overall survival probabilities than the 31 patients who had failed to achieve at least a minor cytogenetic response, namely 89.5% *versus* 63.6% (P=0.002), 100% *versus* 74.4% (P=0.0007) and 100% *versus* 76.8% (P=0.0005), respectively (Figure 5).

At 6 months 78 patients remained in chronic phase. The 40 patients who had a major cytogenetic response had better event-free, progression-free and overall survival probabilities than the 38 patients who had failed to achieve a major cytogenetic response, namely 90.3% *versus* 75.0% (P=0.03), 100% *versus* 79.2% (P=0.006) and 100% *versus* 84.2% (P=0.01), respectively. At 6 months 43 patients had a *BCR-ABL/ABL* ratio of 15% or less. In our laboratory 90% of patients with a ratio of 15% or less for whom simultaneous cytogenetic samples are available are in major cytogenetic response. These 43 individuals had better event-free, progression-free and overall survival probabilities than the 35 patients who had failed to reach

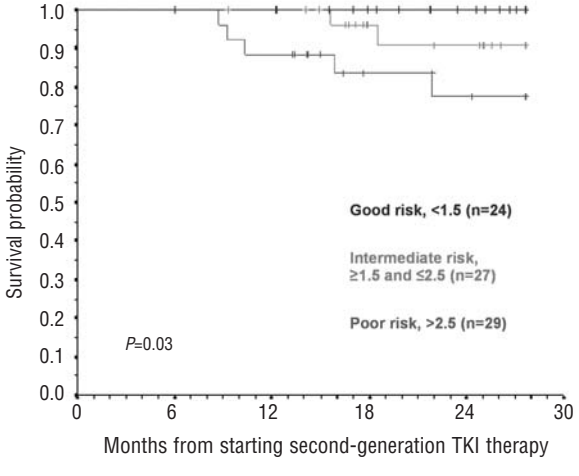

Figure 4. Patients' overall survival according to the Hammersmith score (see text). The top line indicates good risk patients, the middle line intermediate risk patients, and the bottom line poor risk patients

that level of molecular response, namely 91.9% *versus* 70.1% (*P*=0.01), 100% *versus* 77.1% (*P*=0.002) and 100% *versus* 82.1% (*P*=0.005), respectively (Figure 5). We performed multivariate analysis for event-free, progression-free and overall survival, including the molecular and cytogenetic responses at 6 months and the variables are shown in Table 1. We found that the achievement of a *BCR-ABL/ABL* ratio of 15% or less at 6 months was the only independent predictor for event-free, progression-free and overall survival.

We also performed a 12-month landmark analysis for event-free, progression-free and overall survival. Patients who were in complete cytogenetic response at 12 months had significantly superior event-free and overall survival probabilities compared to patients who had failed to achieve a complete cytogenetic response, namely 97.3% *versus* 79.8%, (*P*=0.04) and 100% *versus* 85.3 (*P*=0.02). No significant differences were found in progression-free survival (*data not shown*).

Development of tyrosine kinase domain mutations and clonal evolution on second-generation tyrosine kinase inhibitor therapy

Five patients developed a mutation during therapy with 2G TKI (E255K, F317L, n=2, T315I and Y253F) in a median time of 6 months, (range, 1.8-14.3). These patients had a significantly worse event-free survival (RR=7.30, *P*=0.002), progression-free survival (RR=10.2, *P*=0.005) and overall survival (RR=7.0, *P*=0.02) than those without detectable mutations. During 2G TKI therapy, four patients developed clonal evolution while otherwise still in chronic phase. These patients also had significantly inferior event-free survival (RR=34.6, *P*<0.0001), progression-free survival (RR=10.7, *P*=0.03) and overall survival (RR=11.1, *P*=0.004).

Discussion

We have shown that responses to 2G TKI can be accurately predicted by using the proposed 'Hammersmith score' (Figure 2). The score is calculated by considering jointly the best cytogenetic response on imatinib, the Sokal risk group and the occurrence of recurrent neutropenia during treatment with imatinib that requires a reduction of the dose of imatinib to below 400 mg/day despite hematopoietic growth factor support. The score discriminates three groups of patients: patients in the good risk group had a 2.5-year cumulative incidence of complete cytogenetic response of 100%, whereas patients in the intermediate and poor risk groups had complete cytogenetic response incidences of 52.2% and 13.8%, respectively. We validated the risk score by applying it to an independent population of patients in whom imatinib had failed.

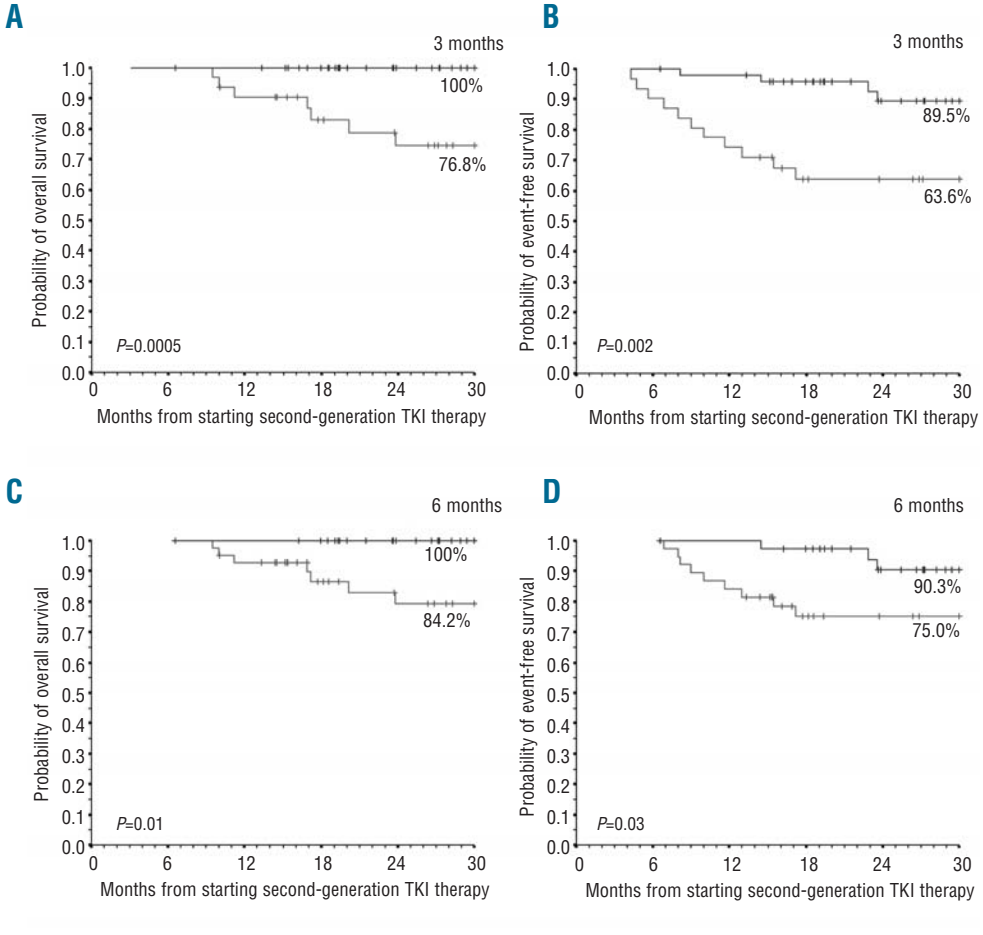

Figure 5. Landmark analyses for overall and event-free survival according to cytogenetic responses at 3 and 6 months. (A) and (B) At 3 months the 48 patients still in chronic phase who had achieved at least a minor cytogenetic response (upper lines) had better overall and event-free survivals (also progression-free survival, see text) than the 31 patients (lower lines) who had failed to achieve a minor cytogenetic response, namely 100% *versus* 76.8% (*P*=0.0005) and 89.5% *versus* 63.6 (*P*=0.002) respectively. (C) and (D) At 6 months 78 patients remained in chronic phase (76 with no "events"). The 40 patients who had achieved a major cytogenetic response (upper lines) had better event-free survival and overall survival (also progression-free survival, see text) than the 38 patients who were not in major cytogenetic response (lower lines), namely 100% *versus* 84.2% (*P*=0.01) and 90.3% *versus* 75.0% (*P*=0.03) respectively. Vertical lines indicate censored patients.

The importance of the predictive factors used in our scoring system has been identified previously. Others reported that the best cytogenetic response to imatinib is an independent predictive factor for cytogenetic response on 2G TKI therapy.[9] We reported previously the predictive value of the Sokal score in patients in late chronic phase who receive imatinib as a second-line therapy,[26] and we highlighted the adverse prognostic implications of cytopenias in imatinib-treated patients.[2,26]

We also found that the time elapsed from first identification of imatinib treatment failure to beginning therapy with a second-generation TKI was a significant independent predictor of lack of complete cytogenetic response (Table 1). This could support the recommendation that patients proven resistant to imatinib 400 mg/day should be started on a second-generation TKI as soon as feasible. However, since, in practice, many patients are not adequately assessed at regular intervals after starting imatinib, we decided not to include this variable in the score.

In 2006 Baccarani *et al.,* on behalf of the European LeukemiaNet, published a series of empirical recommendations designed to help clinicians identify CML chronic phase patients responding poorly to imatinib.[23] The recommendations were based on response to treatment at various time-points assessed using specific criteria. Our data suggest that the same criteria or criteria similar[27] to those used for patients receiving front-line imatinib therapy might be used to define treatment failure in patients given 2G TKI after failure of imatinib, with assessment points at 3, 6 and 12 months after starting the new drug. For example, we found that patients who failed to achieve a minor cytogenetic response at 3 months or a major cytogenetic response at 6 months had significantly worse event-free, progression-free and overall survival probabilities and a lower probability of achieving a complete cytogenetic response than patients who did achieve the aforementioned responses at 3 and 6 months. Although more patients and longer follow-up are required before a formal recommendation can be made, our data suggest that for patients on 2G TKI therapy who fail to achieve a minor cytogenetic response at 3 months, major cytogenetic response at 6 months or complete cytogenetic response at 12 months, the therapeutic strategy needs to be reassessed.

It is possible that molecular monitoring could be as or sometimes more informative than cytogenetic studies. We found that patients on second-generation TKI who had a *BCR-ABL/ABL* ratio of 15% or less (15.3% on the international scale) at the 6-month landmark analysis had significantly better event-free, progression-free and overall survival probabilities than those with higher ratios. Furthermore the molecular response was the only significant independent prognostic variable. However the fact that transcript values obtained in different laboratories are not yet easily compared may limit the general utility of this technique to define responders in the early stages of therapy, although we are aware that efforts to achieve international standardization are well advanced.[28] We previously reported that finding kinase domain mutations in patients treated with imatinib who do not show any other signs of resistance is associated with a poor prognosis.[21] We have now confirmed these results in patients treated with 2G TKI.

Finally our data contribute to the vexed issue of whether to treat patients in whom imatinib has failed with a 2G TKI or stem cell transplantation (assuming that they have a suitably matched donor). Patients with a low Hammersmith score may be expected to benefit from dasatinib or nilotinib therapy. Patients with a high Hammersmith score could be candidates for stem cell transplantation, particularly if they can be classified, according to standard criteria, as having a *good risk* of surviving a transplant procedure.[29,30] Patients with an intermediate or good risk Hammersmith score or patients classified as *poor risk* for transplantation could be treated with 2G TKI; their cytogenetic responses at 3 or 6 months could be used to assess the need to maintain or change this therapeutic strategy.

Authorship and Disclosures

DMi, JFA, TH, PS, MD, MB, and KR: provided patient care and commented on the manuscript; EN: provided patient care, collected data and commented on the manuscript; RS: revised the statistical analysis and commented on the manuscript; LF: supervised the day-to-day running of minimal residual disease analysis; AR: performed the cytogenetic studies and commented on the manuscript; JSK: performed the molecular studies, assembled the molecular data and commented on the manuscript; HdL: collected clinical data, provided patient care and commented on the manuscript; CP: collected data and commented on the manuscript; JMG: wrote the manuscript. DMa: designed the study, performed the statistical analysis, supervised patient care and wrote the manuscript.

DM received lecture fees from Novartis and Bristol-Myers Squibb; JA received honoraria and lecture fees from Novartis and Bristol-Myers Squibb; JG received lecture fees from Novartis and Bristol-Myers Squibb; DM received honoraria and lecture fees from Novartis and Bristol-Myers Squibb

The other authors reported no potential conflicts of interest.

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