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# Hammersmith score application identifies chronic myeloid leukemia patients with poor prognosis before treatment with second-generation tyrosine kinase inhibitors

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**In this study, we confirm the validity of the proposed Hammersmith score, which identifies three risk categories of patients and establish its strength on a large group of 128 chronic myeloid leukemia patients treated with second-generation tyrosine kinase inhibitors (TKIs) after being resistant to imatinib. Sixty-one patients were identified as good risk group, 27 patients as intermediate risk group, and 40 patients as poor risk group. The 1-year cumulative incidence of complete cytogenetic response was 73% in good risk patients, 40% in intermediate risk patients, and 22% in poor risk patients ($P = 0.0001$). Event-free survival at 3-year was 89% in good risk group, 70% in intermediate group, and 54% in poor risk group ($P = 0.0001$); the estimated 3-year progression-free survival was 95% in good risk category, 93% in intermediate risk category, and 87% in poor risk category ($P = 0.05$). Kaplan–Meier estimated that the 3-year overall survival was 100% in good risk category, 93% in intermediate risk category, and 82% in poor risk category ($P = 0.04$). In conclusion, some prognostic factors before starting second-generation TKIs might predict cytogenetic response and outcome. The so-called Hammersmith score was not yet validated in large series of patients: we demonstrated that this score is able to discriminate patients at high risk of failure and consequent progression before treatment with second-generation TKIs.**

Imatinib mesylate is currently the standard of care for patients with Philadelphia-positive chronic myeloid leukemia (Ph+ CML), as it has demonstrated to induce a complete cytogenetic response (CCyR) in up to 90% and a major molecular response (MMR) in 40–60% of patients [1]. However, the emergence of imatinib resistance has become a relevant problem, and BCR-ABL mutations are the most frequent mechanism underlying this phenomenon, together with other not well-known mechanisms of resistance [2]. Multiple strategies have been developed to overcome imatinib resistance, including drug dose escalation, combined treatments and novel targeted agents, such as nilotinib and dasatinib [2]. Also with second-generation tyrosine kinase inhibitors (2° TKIs), the achievement of CCyR is associated with long-term survival, and it is extremely important to define as early as possible the likelihood of response to these drugs [3]. A score aiming at early identification of CML patients showing sensitivity to 2°TKIs was proposed by the Hammersmith group [4]. The score was created by analyzing 80 patients and was based on three prognostic factors: previous cytogenetic response to imatinib, Sokal risk at diagnosis, and recurrent neutropenia during imatinib. Aim of our study was to confirm the validity of this score and to establish its strength on a large group of CML patients resistant to imatinib and treated with 2°TKIs.

**TABLE I. Patient Characteristics According to Hammersmith Stratification at 2° TKI Start**

| | Good risk (61 points) | Intermediate risk (27 points) | Poor risk (40 points) |
|---|---|---|---|
| Age (years, median) | 52 | 56 | 48 |
| Sex (M/F) | 40/21 | 10/17 | 19/21 |
| Sokal risk at diagnosis | | | |
| Low | 37 (59%) | 10 (37%) | 19 (45%) |
| Intermediate | 16 (26%) | 9 (33%) | 16 (40%) |
| High | 8 (13%) | 8 (29%) | 5 (12%) |
| Additional cytogenetic aberrations (ACA) in Ph+ cells | 4 | – | 2 |
| del(9q) | – | – | 1 |
| Baseline mutations | 3 (F359V, E255K, D276G) | 3 (M351T, F359V, M244V) | 5 (E292K, Q288R, Y253H, M351T, F317L) |
| Type of imatinib resistance | | | |
| Primary | 13 | 13 | 8 |
| Acquired | 48 | 14 | 32 |
| Type of 2° TKI | | | |
| Nilotinib | 18 | 9 | 13 |
| Dasatinib | 42 | 18 | 27 |
| Patients received allogeneic hematopoietic stem cell transplant | – | 1 | 4 |

**TABLE II. Outcome of Patients Grouped According to Hammersmith Score**

| | Good risk (61 points) | Intermediate (27 points) | Poor risk (40 points) | *P* |
|---|---|---|---|---|
| CCyR, 1 year (%) | 73 | 40 | 22 | 0.001 |
| MMR (%) | 52 | 28 | 13 | 0.001 |
| EFS, 3 year (%) | 89 | 70 | 54 | 0.0001 |
| PFS, 3 year (%) | 95 | 93 | 87 | 0.05 |
| OS, 3 year (%) | 100 | 93 | 82 | 0.04 |
| Death | – | 2 | 7 | 0.03 |
| Blast crisis/events | 2/7 | 2/7 | 4/7 | ns |

Table I shows clinical–biological features of patients at the time of starting second-generation TKIs. According to Hammersmith criteria of stratification, we identified 61 patients with good risk, 27 patients with intermediate risk, and 40 patients with poor risk. Well-balanced clinical features were found among the three groups, with respect to median age and Sokal's risk at diagnosis. Only six patients with additional cytogenetic abnormalities were detected, four in the good risk category and two in the poor risk group, whereas only one patient had del(9q) at presentation. Mutational screening before starting second-generation TKIs was performed in all patients and three mutations in good risk patients (F359V, D276G, E255K), three in intermediate risk group (M351T, F359V, M244V), and five in poor risk patients (E292K, Q288R, Y253H, M351T, F317L) were detected. Four patients developed a T315I mutation during treatment with second-generation TKIs (one patient in good risk group, one patient in intermediate and two patients in poor risk group). Primary resistance was the main cause of switch to second-generation TKIs in 34 patients (16 patients in good, 16 patients in intermediate, and eight patients in poor risk), whereas acquired resistance was detected in 94 patients, mostly in the good and poor risk categories. Forty patients received nilotinib as second line while 87 patients were switched to dasatinib. The overall rate of CCyR in this series was 57%: the rate of CCyR obtained with dasatinib was 64% and with nilotinib 55% ($P$ = ns). The absence of differences allowed us to consider a single cohort, although treated with two different drugs. Table II shows response and outcome of patients grouped according to Hammersmith score: 1-year probability of achieving CCyR was 73%, 40%, and 22%, in good, intermediate, and poor risk groups, respectively ($P = 0.001$). We assessed in a landmark analysis the prognostic weight of cytogenetic response in all the identified categories, but we did not find a different outcome for patients at high risk with achievement of MCyR at 12 months. With this score, we also identified a difference in MMR rate: 52% in good risk category, 28% in intermediate, and 13% in poor risk categories ($P = 0.001$). Statistical differences were found in 3-year event-free survival (EFS; 89%, 70%, and 54%, respectively) ($P = 0.0001$), a trend of significance in 3-year progression-free survival (PFS) (95%, 93%, and 87%, $P = 0.05$) and in 3-year overall survival (OS) (100%, 93%, and 82%, respectively, $P = 0.04$, Fig. 1). Nine patients died during the follow-up, eight for progression of disease to blast phase and one patient for unrelated CML cause.

After imatinib failure, the availability of second-generation TKIs has provided new therapeutic strategies, replacing allogeneic transplantation as second choice. Nilotinib and dasatinib allowed the achievement of cytogenetic response in more than 60% of patients, with complete response in about 50%, with estimated EFS and OS being 50–60% and 80–85%, respectively [3]. From phase II trials with either drugs it appeared that better survival rate was observed in patients who experienced cytogenetic relapse during imatinib treatment instead of hematological relapse or primary resistance to imatinib, after a median follow-up of 2–3 years. An independent analysis outside of clinical trials was published from MD Anderson Cancer Center, to determine when patients with incomplete response on second-generation TKIs should be considered for alternative treatments. The outcome of 113 patients treated with nilotinib or dasatinib was analyzed: after 12 months, the achievement of major cytogenetic response displayed a survival advantage compared to minor cytogenetic response or complete hematologic response only. The authors suggested that patients with no cytogenetic response at 3 and 6 months should be considered for alternative therapies [5]. Another study by the same group [6] showed that long-term outcome of patients who failed imatinib and were treated with second-generation TKI may be predicted by the in vitro sensitivity of BCR-ABL1 kinase domain mutations. They assessed

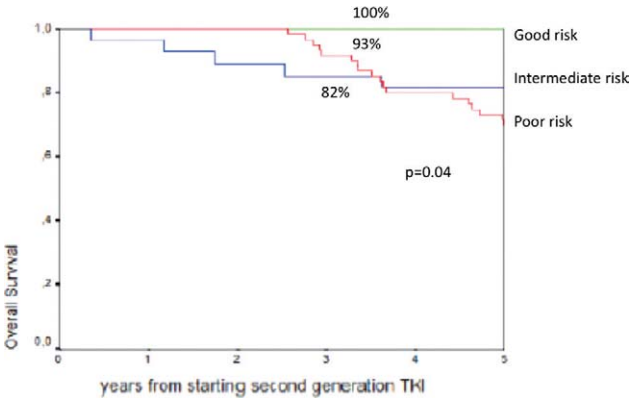


Figure 1. Overall survival according to Hammersmith stratification. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that scoring mutation based on in vitro inhibitory concentration of each TKI mutation can predict long-term clinical outcome. Forty-one patients out of 169 treated with dasatinib and 45 out of 169 treated with nilotinib were found to have a mutation. Inhibitory concentration values for each TKI mutation were stratified into high ($n = 42$), intermediate ($n = 25$), low (T315I, $n = 9$), or unknown sensitivity ($n = 10$). Hematologic and cytogenetic response rates were similar for patients with or without mutations. For patients in chronic phase (CP), hematologic and cytogenetic responses correlated with mutation score. A worse EFS and OS were observed in patients with intermediate and low mutation scores [6]. Hughes et al. [7], to determine whether response to the second-generation TKIs can be predicted, monitored 155 patients in CP who were treated with nilotinib ($n = 73$) or dasatinib ($n = 82$; all treated with 100 mg or more per day, with 76 of these receiving 70 mg twice daily) after imatinib failure for a median of 18 months (range 3–36 months). They found that BCR-ABL ratio measured at 3 months was predictive of achievement of MMR at 24 months. Patients with a BCR-ABL value of $\leq$1.0% International Scale (IS) had an 86% probability of achieving an MMR by 24 months, compared to patients with values of 1–10% IS or $>$10% IS that had a probability of MMR of 55% and 4%, respectively [7]. The assessment of prognostic factors before treatment with second-generation TKIs and the identification of patients with unfavourable features that may predict failure to second-line therapy remain major current problems. Recently the Hammersmith group identified three factors that are associated to achievement of CCyR with dasatinib or nilotinib second-line [4]. Sokal risks at diagnosis, best cytogenetic response obtained on imatinib, occurrence of neutropenia at any time during imatinib therapy (which required dose reduction and/or use of growth factor support) were selected as independent predictor factors of response to second TKIs. Also the time from detection of imatinib failure to start of second TKI was recognized as an independent prognostic factor, but was not included in the final analysis due to the difficulty to have this data in all patients. The devised score was then validated in a small cohort of 28 Scottish patients. We could evaluate the score in all consecutive patients treated with second-generation TKIs, because all patients were followed from diagnosis. Instead, in a recent publication aimed to identify a prognostic model for prediction of response, the MD Anderson Cancer Center was not able to test the Hammersmith score due to the fact that 61% of patients were referred to the center only after ima-

tinib failure and they could not obtain accurate information on Sokal risk at diagnosis, neutropenia during imatinib therapy, and exact time elapsed from start imatinib to development of imatinib failure and start of 2° TKI [8]. However, the authors, who proposed another score based again on clinical features, stated that the time from which the patient was taken off imatinib to second-generation TKIs start was not identified as independent factor for EFS. Although one of the reason of choice of second-generation TKIs is the occurrence of mutations at the moment of resistance and the incorporation of this data may increase the prognostic ability to identify patients with poor outcome, this information is not easily available or, as in our series, not representative of all mechanisms of resistance. Our study represents a validation of the proposed Hammersmith score, and we found that it is easily applicable, although this implies that an adequate monitoring during imatinib therapy has been performed. Patients considered at poor risk should be closely monitored during second-generation TKIs, and young patients with a sibling donor should be alerted for a possible transplantation as therapeutic option, whereas patients with no available donor and/or presence of comorbidities should be candidate to alternative options that need to be investigated.

### Methods

Patients were enrolled in this study from seven Italian centers. All were in CP, resistant to imatinib and then treated with 2° TKIs, nilotinib or dasatinib, outside of clinical trials or enrolled in phase II and III studies aimed to prove the safety and efficacy of the drugs in resistant and/or intolerant patients to imatinib [9,10]. Chronic phase was defined according to WHO definition. Conventional cytogenetic analysis was performed on bone marrow cells by G-banding technique and at least 20 metaphases were analyzed on direct and short-term cultures after 24 hr; analyses were performed every 3–6 months. For molecular investigations, mononuclear cells were isolated from 20 mL peripheral blood after separation on a Ficoll-Hypaque gradient. RNA extraction, real time-PCR, and real time-quantitative polymerase chain reaction were performed as already described [11]. BCR-ABL1/ABL1 ratio was measured using RQ-PCR, as previously described, and the results were referred to IS [12]. For mutational screening, the methodology used for mutation detection was the following: after RNA extraction and reverse transcription, overlapping fragments covering the entire kinase domain were generated by nested PCR and screened by denaturing high performance liquid chromotography. In positive cases, a direct sequencing was performed. Responses to imatinib standard dose were defined according to European LeukemiaNet recommendations [13]. Failure was considered as the lack of complete hematologic response at 3 months and of cytogenetic response at 6 months, the attainment of less than partial cytogenetic response at 12 months, of less than CCyR at 18 months, or the loss of complete hematologic response, CCyR or acquisition of BCR-ABL mutations and clonal cytogenetic evolution (abnormalities in Ph+ cells) at any time. Suboptimal response was defined by the lack of cytogenetic response at 3 months, less than partial cytogenetic response at 6 months, partial cytogenetic response at 12 months and less than MMR at 18 months, or mutations of BCR-ABL or loss of MMR at any time points.

Statistical analysis was carried out using the SPSS software package. Probabilities of OS, EFS, and PFS were calculated using the Kaplan–Meier method. PFS was defined as survival without evidence of accelerated or blastic phase during therapy with second-generation TKIs. EFS was considered as survival without death from any cause, loss of haematological or cytogenetic response (major or complete), progression of disease.

The Hammersmith model was calculated by allocating points as described by Milojkovic et al [4]: (a) CCyR on imatinib, 0 points; 1–94% Ph-positive metaphases, 1 point; 95% or more Ph-positive metaphases, 3 points; (b) Sokal risk group: low, 0 points, intermediate or high, 0.5 points; (c) neutropenia during imatinib treatment: no neutropenia, 0 points; recurrent episodes of grade 3–4 neutropenia that required dose reduction, 1 point. Patients were divided into three categories: good risk, patients with assigned score of less than 1.5; intermediate risk, patients with score between 1.5 and 2.5; poor risk, patients with score higher than 2.5 [4].

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