Prognostic Factors in Chronic Myeloid Leukemia (CML)

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Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells, arising from a translocation t(9;22)(q34;q11) leading to a juxtaposition of the ABL gene from chromosome 9 and the BCR gene from chromosome 22. The resulting BCR-ABL fusion gene codes for BCR-ABL transcripts and fusion proteins with unregulated tyrosine kinase activity. The translocation also generates a reciprocal ABL-BCR fusion on the derivative chromosome 9 [der(9)] that is transcribed in approximately 70% of cases, although its biologic significance is not known. Several studies have found deletions at or encompassing the ABL-BCR junction in approximately 10–15% of CML cases that almost certainly arise during the translocation process. The molecular pathogenesis of CML is well understood, but the mechanism that leads to the gene translocation is unknown. Genetic instability as a consequence of the BCR-ABL fusion might be responsible for additional chromosomal aberrations or mutations frequently seen in blast crisis.

The definition of prognostic factors is important (i) to select the optimal treatment, (ii) to develop risk-adjusted treatments, (iii) to adjust imbalances of treatment groups in clinical trials, and (iv) to compare outcomes of different studies. Two sets of prognostic factors can be established, namely those that can be identified prior to therapy (baseline factors) and those that can be employed during the treatment (time dependent or response related factors). Most important is accurate identification of the phase of the disease. In early chronic phase important prognostic information is derived from clinical and laboratory features. Currently, the Hasford score [1] is the best predictor of outcome for patients with CML treated with interferon alpha (IFN) and outperforms the older Sokal score [2] in this context. The Hasford score is calculated according to the formula: Score = (0,6666 × age [0 when age <50 years; 1, otherwise] + 0,0420 × 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 × 10⁹/l; 1, otherwise]) × 1000. The score value of individual patients can be calculated

by accessing the website www.pharmacoepi.de. Cytogenetic response is considered the most important time dependent prognostic factor of CML patients after IFN [3] and imatinib [4] therapy. Hasford et al. presented an analysis on IFN treated patients suggesting that cytogenetic response alone is not a valid surrogate marker, as it is dependent on the baseline prognostic profile. The combination of risk group and cytogenetic response does, however, provide useful clinical information [5].

Cytogenetic changes such as deletions of the derivative chromosome 9 also affect prognosis. Importantly, deletions have been associated with an adverse prognosis, at least for cases treated with hydroxyurea or IFN-based therapies [6, 7]. However, not all studies have found that der(9) deletions are an indicator of inferior outcome. Imatinib might partly overcome the negative prognostic effect of these deletions, but size and location of the deletions seem to matter. Deletions of downstream BCR sequences at the der(9) fusion junction were first identified in a minority of patients with CML by Southern blot analysis [8]. Development of fluorescent *in situ* hybridization (FISH) probes revealed that the deletions were larger than had been previously suspected and frequently included loss of SMARCB1, 500 kb telomeric of BCR [9]. Subsequently, it was shown that the deletions were variable in size, often encompassed several megabases, and usually included both chromosome 9- and chromosome 22-derived sequences, i.e. they spanned the reciprocal ABL-BCR junction [10]. This study was also the first to suggest that the deletions may be associated with a poor prognosis, a hypothesis that was expanded in a larger analysis from the same group in which the median survival of the 14% of cases with deletions (most of which spanned the ABL-BCR junction) was significantly shorter than of those without deletions [6]. The adverse prognosis was confirmed in an independent large study in which patients with deletions had a shorter duration of chronic phase, inferior survival, and increased probability of relapse after stem cell transplantation [7]. A more recent study of IFN treated cases,

however, failed to detect any difference in clinical outcome between cases with and without deletions, although patients with deletions were found to present with significantly lower hemoglobin levels and higher leukocyte counts [11]. Using a novel DNA based MLPA deletion assay Kreil et al. [12] investigated 339 patients enrolled in 3 trials of the German CML Study Group in over 15 years with an observation time of up to 16 years. This was the largest group of IFN treated cases to be analyzed for the impact of deletions. The proportion of deletions was comparable to other studies, but only a relatively small proportion of deletions (36% of cases with deletions; 6% of all cases) spanned the ABL-BCR fusion point. Although they found no difference in survival between cases with and without deletions, more detailed analysis indicated that deletions that spanned the fusion point were a significant indicator of inferior prognosis. Current models suggest that the poor prognosis associated with deletions is probably the result of heterozygous loss of one or more loci rather than loss of ABL-BCR expression, general genomic instability, or effects on BCR-ABL [13–15]. These findings are consistent with the hypothesis that at least two separate loci may be targeted, one on each side of the fusion junction. However, it is not clear how deletion of both these loci could result in an adverse prognosis, whereas deletion of just one has the opposite effect. Multivariate analysis confirmed that at diagnosis, deletion status provides independent, statistically significant prognostic information with respect to survival probability. However, only large deletions that span the der(9) breakpoint are an adverse risk factor.

In the study by Li et al. [16] published in this issue of *ONKOLOGIE*, FISH analysis was used to assess der(9) deletion status of 48 CML patients in blast crisis. Among the 48 CML patients, 8 (16.7%) showed der(9) deletions, and the deletions had also existed at diagnosis. The median duration of chronic phase for patients with der(9) deletions was 18 (range 4–38) months compared to 48 (range 0–204) months for patients without deletions ($p < 0.001$). der(9) deletions were not associated with increased karyotypic instability. There was no difference in the probability of the der(9) deletions between the cases which progressed to myeloid and lymphoid blast crisis. These results showed that, following hydroxyurea therapy in chronic phase, patients with der(9) deletions had a shorter duration of chronic phase, and the deletion status influenced the outcome of treatment with hydroxyurea. However, size and location of the mutations were not analyzed.

Standard therapy for newly diagnosed CML patients is imatinib 400 mg/day [4]. After imatinib therapy, der(9) deletions have not yet been reported to be a strong prognostic indicator. Huntly et al. found no difference in survival between patients with and without deletions, although deleted cases had more rapid disease progression and exhibited poorer hematologic and cytogenetic responses [17]. Quintas-Cardama et al., how-

ever, described no influence of der(9) deletions with regard to response, survival, or response duration [18]. It seems that much longer follow up will be required to determine whether deletions do or do not have prognostic value for imatinib treated patients.

Using imatinib therapy, the relative risk, either by the Sokal [2] or Hasford [1] methods, predicts the cytogenetic response to 400 mg imatinib daily [4, 19]. Moreover, the Sokal risk score has been reported to predict also molecular response and overall survival. In imatinib treated patients, the overall survival at 54 months was 94, 88, and 81% for low, intermediate, and high Sokal risk patients, respectively ($p < 0.001$) [4]. These risk definitions, which were derived from patients treated with conventional chemotherapy or IFN, are still useful, and should be used until further studies identify and confirm other factors of possible prognostic relevance, such as genomic profile [20–23], genetic polymorphisms [24], Wilms tumor gene expression [25], total phosphotyrosine levels in CD34+ cells [26], and the phosphorylation level of the adaptor protein Crkl [27]. Additional chromosomal aberrations, including Ph duplication are also candidate adverse prognostic factors.

Early cytogenetic response to imatinib seems to be the most important response-related prognostic factor [28–30]. If no cytogenetic response is achieved after 3 months, there is still a 50% chance of achieving a complete cytogenetic response (CCR) later on. If there is any (even minimal) cytogenetic response after 6 months of treatment, there is still a fair chance of achieving a CCR later on, but if the 6-month karyotype remains 100% Ph⁺, the probability is only 15%. After 12 months of treatment, if the cytogenetic response is partial, the probability of achieving a CCR at 2 years is still 50%, but if the response is less than partial, this probability becomes less than 20%.

The level of molecular response was also found to be an important dynamic factor of prognosis. It was reported that transcript levels after 1 or 2 months of imatinib treatment predicted late responses [28, 29], that a low level of residual disease was associated with continuous remission [30], and that a major molecular response after 12 months of treatment was associated with a better event and progression free survival [19].

Despite lack of data on the size and location of the mutation the study reported here demonstrated a significant difference in the time to blast crisis in the natural course of CML in patients treated with hydroxyurea only. The study also shows the limitations of hydroxyurea therapy with a rapid natural evolution of the disease from chronic phase to advanced disease [15]. Obviously, imatinib therapy does override the negative impact of such mutations. For imatinib therapy, other molecular factors identifying early relapse or transformation, should be detected using molecular methods in combination with large prospective controlled clinical trials.

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