Conventional and novel tools for defining the risk of the individual patient with chronic myeloid leukemia and for monitoring treatment

Since chronic myelogenous leukemia (CML) was shown to be associated with a specific chromosomal translocation, t(9;22)(q34;q11), which generates the Philadelphia (Ph) chromosome and the hybrid BCR-ABL gene,\(^1,2\) these specific cytogenetic and molecular alterations have constituted the basis for therapy surveillance of the disease and, to this purpose, novel and more sophisticated tools have been progressively introduced into clinical practice. Though some notions that emerged from the huge number of studies performed on this subject are universally accepted, there are still aspects which appear controversial and that will require further investigation.

It is already clear, particularly for patients treated with interferon-\(\alpha\) (IFN-\(\alpha\)), the first biological agent capable of inducing cytogenetic remission in patients with CML, that the degree of tumor load reduction during therapy is an important prognostic factor for CML patients.\(^3\) However, the hematologic response, which is achieved with the normalization of peripheral blood counts and absence of signs and symptoms of disease and which corresponds to a 1 log reduction in the leukemia burden, does not represent per se a sufficient therapeutic goal in CML, as patients in hematologic remission but who are still 100% Ph-positive invariably progress to a blastic phase and die from its complications. In contrast, the degree of cytogenetic remission, which, if complete, indicates an approximately 2 log reduction of leukemia cell load, has been shown to represent a strong prognostic indicator and it has often been suggested in clinical trials as a possible surrogate marker for overall survival.\(^3\) The cytogenetic response is established on the basis of the proportion of residual Ph-positive metaphases and is defined as complete (0% of Ph-positive metaphases), partial (1-33%), minor (34-66%), or minimal (67-99%), whereas a major response represents the sum of the complete and partial cytogenetic responses. Only major (complete and partial) cytogenetic remissions have been shown to be associated with an increased survival, whereas the impact of minor or minimal cytogenetic responses on prognosis remains negligible.\(^3\)

Finally, molecular remission was traditionally defined on the basis of the detection of residual BCR-ABL transcripts by conventional qualitative nested reverse transcriptase polymerase chain reaction (RT PCR). Indeed, data on the prognostic significance of achieving a molecular remission as defined above, have been obtained mainly in cohorts of patients subjected to allogeneic bone marrow transplantation, the only category of patients able to achieve this condition in a consistent percentage of cases.\(^4\) For all the other patients treated in a different way and in particular for those treated with IFN-\(\alpha\), in whom the number of absolute molecular remissions in terms of persistent polymerase chain reaction (PCR) negativity was very

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References

low, the gold standard for evaluating a patient’s response to treatment remained conventional cytogenetic analysis (CCyR), as the simple non-quantitative RT PCR analysis was too sensitive to discriminate sufficiently between patients in whom the residual volume of disease could be in a range of 4 logs. Peripheral blood fluorescence in situ hybridization (FISH) for the BCR-ABL translocation was also reported to be an easy and sensitive method for serial monitoring of CML patients and is the only method able to reveal the presence of deletions on the derivative chromosome 9q+, whose presence has been reported to be associated with a worse prognosis for patients treated with IFN-α, but has not been able to replace the more widely used conventional cytogenetic methods.

Two factors have recently changed this picture: the development of simple and reliable methods for quantitative PCR and the clinical introduction of the tyrosine kinase inhibitor, imatinib mesylate, in the therapy of CML.

Even in the past, in view of the very limited value of qualitative PCR, several groups had developed quantitative PCR assays based on competitive PCR strategies to estimate the amount of residual disease in patients able to achieve a complete cytogenetic remission, but who remained RT PCR-positive. The data obtained showed that the level of minimal residual disease correlated with the probability of relapse in complete cytogenetic responders to IFNα as well as in patients who underwent allogeneic bone marrow transplantation. In the latter group, competitive PCR was also used to adapt treatment and to determine the optimum time to initiate donor lymphocyte infusion and to monitor the response. However, the competitive PCR methods are labor intensive, time-consuming, difficult to standardize and not suitable for large-scale analyses. More recently, real-time quantitative RT-PCR (RQ PCR) assays have been developed to monitor the kinetics of residual BCR-ABL transcripts over time. Variables in the quantitative PCR assay (quality and quantity of the RNA and the reverse transcription step) may be controlled by quantification of transcripts of a control gene (ABL, G6PD or β2-microglobulin) as an internal standard. Moreover, standardization and the introduction of rigorous, internationally accepted controls have been established to enable RQ PCR to become a robust and routine basis for therapeutic decisions. These advances are particularly needed in light of the extremely positive therapeutic results obtained from using imatinib mesylate in CML therapy. In fact, about 75% of patients with newly diagnosed chronic phase CML treated initially with imatinib achieve CCyR and imatinib also induces Ph negativity, though less frequently, in patients treated in advanced phases of the disease.

Very recent studies show that the amount of residual disease at 12 months, established by RQ PCR in terms of log reduction of the BCR-ABL transcripts with respect to the pre-therapy copy number, is statistically significant in predicting the risk of disease progression for newly diagnosed CML patients achieving CCyR under imatinib therapy. CML patients resistant to or intolerant of IFN, who subsequently obtained a CCyR with imatinib therapy, have also been demonstrated to have a lower risk of losing CCyR and RQ PCR analysis reduction in the amount of BCR-ABL transcripts.

Other important conclusions recently reached were that early reduction of BCR-ABL mRNA transcript levels predicts cytogenetic response in CML patients treated with imatinib and that this parameter can also identify groups of patients with different risks of progression as the incidence of progression, defined by

![Figure 1. Relationship between the leukemic load in chronic myelogenous leukemia (CML) and the commonly used methods of analysis that allow to establish its reduction.](image-url)
hematologic, cytogenetic or quantitative PCR criteria, was significantly higher in patients who failed to achieve a 1 log reduction by 3 months or a 2 log reduction by 6 months.21

From the practical point of view, as comparable results of RQ PCR analysis can be obtained in bone marrow (BM) or peripheral blood (PB) samples and a good correspondence between the levels of BCR–ABL transcripts and the degree of cytogenetic response has been reported in many studies, can we expect that RQ PCR analysis could completely replace conventional cytogenetics in therapy surveillance of CML patients?

In this issue of the journal, an elegant paper by Toralf Lange and colleagues22 clearly concludes that RQ PRC should not replace conventional cytogenetics for monitoring CML patients, at least during the early phase of imatinib therapy. In their work, the proportion of Ph-positive metaphases at 3 months emerged as the only independent factor that predicted major cytogenetic remission (MCyR) at 6 months and, more importantly, progression-free survival (PFS) at 2 years in a multivariate analysis that considered several pre-therapy characteristics of the CML patients as well as the RQ PCR data at 3 months. The reason for this rather surprising finding is not clear at the moment, but the authors speculate that cytogenetics analysis measures the residual proliferative potential of the Ph-positive cells under imatinib therapy and that this parameter more closely reflects the intrinsic nature of the disease and reflects the sensitivity to imatinib more accurately than does the simple level of BCR–ABL transcripts.

Beside these important findings, even considering that RQ PCR is fundamental for monitoring patients with CCyR, there are other reasons suggesting that cytogenetics should not be completely replaced by RQ PCR in the follow-up of CML patients. Additional chromosomal abnormalities present at diagnosis or arising during the disease may have a prognostic influence. Recently, several studies also reported the occurrence of clonal cytogenetic abnormalities in the Ph-negative cells, which appeared after suppression of the Ph-positive clone by imatinib23 and, in a minority of cases, could also lead to the appearance of a myelodysplastic hematopoiesis.24

In conclusion, the impressive overall success recently obtained with the use of imatinib in CML therapy, must not mask the fact that a small, but substantial, percentage of patients may still benefit from a further refinement of the therapeutic conduct, which may depend on the acquisition at diagnosis and/or during therapy of a set of information generated by different types of analysis, all of which may be important to define the risk of the individual CML patient precisely. The cost of these analyses represents only a small percentage of the global cost that the clinical and thera-
peutic management of a CML patient generally requires and, therefore, not only from a human but also from a merely financial point of view, it would be silly to jeopardize the success of the entire therapeutic plan to save the cost of a single analysis sometimes helpful in redirecting the therapeutic strategy.

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References
Chronic lymphocytic leukemia in 2003

During the last decade, there has been a resurgence of interest in research about chronic lymphocytic leukemia (CLL). An understanding of the molecular basis of this hematologic malignancy has led to the appreciation that several different B-cell diseases are represented under this name.

Several lines of data now suggest that B-cell chronic lymphocytic leukemia may actually be two diseases, reflecting the mutated and unmutated state of the immunoglobulin heavy-chain gene. The current use of fluorescent in situ hybridization permits a more accurate evaluation of the cytogenetics of the malignant cells, identifying distinct subsets of patients with strong correlations between the chromosome abnor-