PROCEEDINGS ARTICLE

Prognostic factors in CLL

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Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease, as some patients progress rapidly toward the more advanced stages, whereas others survive for a long period without the need for treatment. This heterogeneity of clinical course was somehow unexplained until studies on the CLL cell features disclosed that the CLL clones were heterogeneous and were characterized by different phenotypic and genotypic features in the different patients. On the basis of these observations, it was determined in retrospective studies that clones characterized by unmutated *IGHV* genes, and/or CD38 and/or ZAP-70 expression conferred a more severe prognosis to the CLL patients. Here, we present data on prospective studies carried out on Binet A-stage patients, in whom the markers were determined at diagnosis and their predictive value was assessed in comparison with chromosomal abnormalities and gene expression or micro RNA profiles. In addition, hypothesis on the potential pathogenetic role of these markers will be presented.

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Chronic lymphocytic leukemia (CLL) is characterized by a very heterogeneous clinical course. In some cases, the disease progresses quite rapidly (i.e., in a few years) toward the most advanced clinical stages, and the patients subsequently die of CLL despite treatment. In other cases, the disease remains indolent or progresses to advanced stages after many years, and the patients may die of causes other than CLL.¹ Because of this heterogeneity of behavior, the current NCI guidelines recommend not to treat the CLL patients until there is clear evidence for progression, based upon the Rai or Binet clinical staging systems.² These recommendations are based on the necessity to avoid exposure to potentially dangerous chemotherapeutic treatments of patients who will have a few probabilities of progressing and of needing treatment. This attitude may change if we had reliable markers capable of predicting the patients' clinical courses.

In the recent years, a number of molecular and cellular markers have become available,³ which can represent disease progression apparently since the early disease stages, thus representing different tools compared with the classical Binet or Rai staging system that, although very valuable, are only capable of measuring the degree of progression of the CLL in a given patient. For example, patients whose cells utilize unmutated *IGHV* genes have a dire clinical course, compared with those whose cells express *IGHV* genes with acquired somatic mutations.^{4,5} Similarly, the expression of ZAP-70 and/or CD38 by a substantial cell number within the CLL clone represents an indicator of potential rapid progression.^{4,6–10} Although these represent the classical and perhaps the most used CLL markers, there are others that have been recently discovered, characterized and proposed as useful tools in the clinical setting.

Most of the available studies on clinical markers were based upon the retrospective analysis of cohorts of patients, that is, the patients were tested for the marker investigated at any time of the disease and the clinical course traced on the clinical records.^{4,7,9,11–16} This approach obviously poses some limitations, as confounding factors may be represented by potential changes in marker expression during the course of the disease. Indeed,

changes in marker expression during disease course and perhaps connected with changes in disease behavior have been reported. On the other hand, because of the obvious limitations in their organization, and the time needed for their completion, the results of prospective studies have become available only in the recent times.

Five years ago, we started an observational study within the Gruppo Italiano Studio Linfomi aimed at recruiting patients at Binet A stage. These patients were investigated for ZAP-70, CD38 and *IGHV* mutational status, and followed up for their clinical courses. The patients were also tested for the most common chromosomal abnormalities typical of CLL. Finally, the patients were tested at intervals to check for potential changes in their phenotypic/genotypic features. This study has provided information on the prospective predictive value of the investigated markers. In addition, it has indicated the potential changes in the marker occurring with disease course and progression. Moreover, it has permitted the formulation of a scoring system, which leads to the stratification of patients into four distinct risks group depending upon the presence of different numbers of unfavorable prognostic markers. Finally, it has made it possible to assess the predictive value of chromosomal abnormalities and compare their prognostic power with that of the molecular and cellular markers described above.

By using the above-described cohort of patients and using gene expression profile analysis in a supervised manner, it has also been possible to distinguish new predictive markers, such as the receptors for IL21¹⁷ and TNF α ,^{18–20} which may help in distinguishing further cases characterized by a favorable or unfavorable prognosis. These markers can be added to those already in use to increase the sensitivity/specificity of the predictive tests to assess the chance of progression.

Cases recruited in the above clinical trial were also studied with the aim of defining the geometry of the molecular lesions underlying the 13q and the 17p cytogenetic abnormalities. Using a single-nucleotide polymorphism analysis approach together with gene expression profile methodology, it has been possible to

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assess the heterogeneity of molecular lesions and evaluate the role that they have in determining progression. In addition, it was possible to determine the alterations in gene expression profiles caused by the presence of the different types of molecular lesions detected. The cases from the Gruppo Italiano Studio Linfomi observational study as well have been subjected to a miRNA analysis²¹ with the aim of detecting a possible correlation between miRNA expression and disease progression. Indeed, by analyzing the miRNA in cases stratified according to the expression of predictive markers and clinical features, it has been possible to identify a CLL subset characterized by a particularly aggressive clinical course, expression of unfavorable markers and a specific miRNA signature.

In conclusion, cellular and molecular marker analysis on a large cohort comprising more than 500 CLL patients has led to the identification of CLL cases that already from the Binet stage A can be predicted to have different clinical courses and can thus be stratified accordingly. In addition, these observations have made it possible to gather information on the pathogenetic value on the possible role of different molecular lesions (or markers) in disease progression and outcome.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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