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## What is gene profiling contributing to the characterization of chronic lymphocytic leukemia



Gene profiling is a technology that allows to assess simultaneously the levels of expression of thousands of genes in a given cell sample using a single chip. This revolutionary technology has witnessed important applications in the study of acute and chronic hematologic malignancies. These can impact on the diagnostic work-up, on the prognostic stratification, on the correlation with the response to treatment and on the design of innovative therapeutic strategies, as well as on a better understanding of the biologic properties of the leukemic cells and of the non-neoplastic accessory cell populations. This innovative technology has been successfully applied to the study of CLL.

In the context of B-cell chronic lymphoproliferative disorders it is well established that each condition has a different gene profile, i.e. different sets of genes are variably expressed in each given condition. This means that CLL has a unique gene expression signature that differs from that of hairy cell leukemia, prolymphocytic leukemia, non-Hodgkin's lymphomas, etc. The evidence that CLL cases may show a mutated or unmutated IgVH gene profile – an event that takes place within the lymph node germinal center – has opened a debate as to whether CLL represents a unique disease or, instead, whether it harbors

under the same name two conditions characterized by a different cellular target of the transforming event. This uncertainty has been clarified by gene profiling studies which have demonstrated that, irrespective of the mutational status of the leukemic cells, CLL is a unique disease as documented by unsupervised gene expression analysis. By supervised analysis, it was instead shown that the mutated and unmutated cases display a set of genes differentially expressed. By gene expression analysis it was also found that one of the genes expressed at the highest levels in the unmutated cases was ZAP-70. This finding opened the way to recognize the ZAP-70 protein as one of the most important prognostic factors for CLL patients.

The unique gene signature of CLL has been recently confirmed within the international MILE (Microarray Innovation in LEukemia) project where it was found that the diagnostic accuracy of gene profiling in CLL is close to 100%. The correlation between gene profiling and the mutational status and ZAP-70 expression on a large series of cases is currently under investigation.

It has also been reported that the cytogenetic/genetic abnormalities most frequently recorded in CLL are associated with the differential expression of a variable set of

genes. In addition, genes differentially expressed have been reported between T-cell subsets from CLL patients and from normal controls, reflecting the multiple phenotypic and functional abnormalities known to be present in CLL.

Defects in BCR signal transduction and/or altered responsiveness to microenvironment signals may play an important role in CLL initiation/progression. Following IgM cross-linking of CLL primary cells, a different responsiveness to BCR stimulation between IgVH germline or mutated cases is observed. Gene expression profiling has highlighted several differentially expressed genes upon stimulation exclusively in germline patients. Cell cycle analysis and proliferation assay confirm that IgM cross-linking induces a significant cell cycle progression exclusively in germline patients, while a significant increase of apoptotic levels is observed only in mutated cases. These results support the hypothesis that response to BCR ligation may play a crucial role in disease progression in IgVH germline cases. A differential expression of genes may be found between CLL patients with stable disease compared to patients with an aggressive clinical course. Furthermore, in the rare CLL cases showing over time (many years) a spontaneous regression of the leukemic clone to a state of hematologic "remission" we have observed that the residual leukemic cells show a peculiar gene expression profile, that partly

overlaps with that observed in patients with stable disease.

From a therapeutic standpoint it will be important to verify whether the degree of response (or non-response) to different treatment modalities may be associated with a different gene expression profile. In addition, gene expression studies have allowed to document that CLL cells express a series of genes towards which second generation tyrosine kinase inhibitors may be active.

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