Incidence and Prognostic Significance of Immunophenotypic Subgroups in Childhood Acute Lymphoblastic Leukemia: The Experience of the AIEOP Cooperative Study

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The role of immunological phenotype in childhood acute lymphoblastic leukemias (ALL) is important in classifying the different origins of ALL, but the identification of different subgroups of clinical relevance is still under discussion. In fact, frequent discrepancies are observed in the prognostic value of immunological subgroups in individual series. A poor outcome was seen in immunophenotype T in the first clinical studies (Greaves et al. 1981), but these results have not always been confirmed and now the T origin of ALL is not considered a prognostic factor (Hammond et al. 1986).

Other immunological markers have sometimes correlated with the negative response to the treatment, in particular the presence of cytoplasmic immunoglobulins (Crist et al. 1989), the positivity for CD20 (Ludwig et al. 1989a) and the coexpression of myeloid antigens (MyAg) in lymphoid blast cells (Sobol et al. 1987). These results have not always been confirmed by other studies. The nonreproducibility of immunological results observed between different series of patients could be due to various factors, but we suggest that the different therapeutic protocols used for the treatment are a very important variable. To overcome these variables we examined, individually and in combination, the immunological features of 892 patients treated by two different regimens during the same period of time by the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP). The analyses of the immunological subgroups were correlated to the therapy response and to the event-free survival (EFS) and the results suggest the possible role of immunophenotype in drawing the future therapeutic protocols.
Materials and Methods

Some 892 children affected by ALL were analyzed for this study; 528 were treated according to 1987 and 364 to 1988 protocols of AIEOP.

Morphological and cytochemical diagnoses of ALL were performed on bone marrow smears using widely adopted criteria (Bennett et al. 1976). Immunological diagnoses were made by indirect immunofluorescence according to previously described methods (Basso et al. 1985). A panel of monoclonal antibodies and heterologous antisera were utilized in immunological diagnoses; CD1/2/3/5/7 and cytoplasmic CD3 (CyCD3) identifying T-lineage ALL; CD10/19/20/24, cytoplasmic (CyIg) and surface immunoglobulins (slg) identifying B-lineage ALL; TdT and HLA-DR, non-lineage-restricted. To identify MyAg, CD11b/13/14/15/33/w65 were employed. All analyses were performed in the center where the patient was initially examined. Results were then reviewed by a committee and the diagnosis confirmed on the basis of the tests carried out. The immunophenotype were considered adequate when at least CD19, 10, 7, CyCD3, HLA-DR, and slg tests were performed. The definition of immunological subgroups was based on the criteria of Nadler et al. (1984).

The AIEOP 1987 protocols were formulated on the basis of CCSG experience (Steinherz et al. 1986). Briefly, there was an induction phase with vincristine, prednisone, daunomycin, and l-asparaginase; the first three drugs were used in the reinforced reinduction phase and maintenance therapy consisted of methotrexate and 6-mercaptopurin with a monthly pulse of prednisone and vincristine (Vecchi et al. 1991).

The AIEOP 1988 protocols were oriented according to those of BFM (Schrappe et al. 1987b), with the induction phase comprising vincristine, prednisone, daunomycin, l-asparaginase, cyclophosphamide, and ara-C, a consolidation phase followed by high-dose methotrexate, and a reinduction phase similar to the initial one. Maintenance therapy was with methotrexate and 6-mercaptopurin (Rossi et al. 1991). The enlistment of patients in the protocols was different; infants with ALL were excluded in the 1987 protocols but included in the 1988 ones. Both protocols were performed in the same period of time, from 1987 to 1990; the 1988 protocols were performed in the eight largest AIEOP centers to evaluate their feasibility, while the 1987 protocols were applied in the remaining centers.

In analyzing the clinical outcome of immunophenotype T and of positivity of CyIg, CD20, and MyAg, we considered as parameters good response to prednisone (less than 1000 blasts/mm$^3$ in the peripheral blood smears after 7 days of prednisone therapy; Schrappe et al. 1987b) the achievement of complete remission after the first induction cycle, and EFS.

Data were collected with patient-oriented and protocol-specific forms. All the information was stored, controlled, and analyzed by VENUS, an integrated software facilities system running on an IBM mainframe at the Italian Interuniversity Computing Center (CINECA). The EFS analysis was