Lymphoproliferative disease: Micro-methods for diagnosis and target therapy
Paolo Coser Bolzano

Micromethods are largely used for diagnosis and risk stratification of lymphoproliferative disease. The vast majority of the diagnostic methods used in the laboratory are micro- or nano-methods and this presentation will underline those concerning B-cell Chronic Lymphocytic Leukaemia (B-CLL) and Multiple Myeloma (MM).

The B-CLL is characterized by peripheral blood monoclonal B-lymphocytosis associated with bone marrow infiltration. The typical immunophenotipic profile is characterized by weak surface monoclonal Ig (SmIg) and expression of surface CD19+, CD20+, CD22+, CD23+ CD19/CD5+ antigens. However, in a variable number of cases the diagnosis of monoclonality could be difficult to prove due to the low density of SmIg on the cellular surface.

The demonstration that B-CLL lymphocytes also express monoclonal cytoplasmic immunoglobulins (CyIg) as shown by a direct immunofluorescence test on blood SmIg negative mononuclear cells (G.Pianezze, P.Coser et al., Blood 1987, 69,1011) definitely clarified these controversial cases.

Today it's possible demonstrate the monoclonality of B-CLL lymphocytes also with a more sophisticated method based on IgH-PCR GeneScan analysis.

The genetic risk profile, necessary to stratify the prognosis and to guide the therapeutic strategy in the single patient, combines classic cytogenetics, interphase FISH and molecular biology for detecting chromosome deletion or alteration or VH genes mutation. All these methods will be presented and discussed.

Multiple Myeloma (MM) is the malignant counterpart of activated B-cells which undergo IgH somatic mutation and isotype switch recombination during the secondary immune-response.

MM is disseminated by circulating monoclonal myeloma precursor B cells, masked as normal lymphocytes and characterized by selective "homing" in the bone marrow where they receive proliferation, differentiation and osteoclast activation signals by Il-6, IL-1, TNF and other cytokines.

With the intent to improve the outcome of high-dose chemotherapy followed by double peripheral autologous blood stem cells transplantation, which is still regarded today as the treatment of choice for younger MM patients, purging methods of the apheresis product were explored in the recent past.

At our Institution the apheresis product for the second transplant was purged using a panel of 4 or 5 mouse monoclonal antibodies against B-cell antigens (CD10, CD19, CD20, CD22 and CD37) and B-cells selected with sheep anti mouse magnetic beads were analyzed. Genescan analysis after CDR III PCR of the isolated B-Cell fraction revealed in 95% of the apheresis product a contamination by monoclonal myeloma precursor B-cell population as compared with the tumor specific CDR III signal previously identified in the diagnostic bone marrow of 19 patients (M.Mitterer, P.Coser et al., British Journal of Haematology 1999,106,737).

The results of this investigation and the correlation between the clonal pattern and the clinical response after sequential chemotherapy will be presented.

Target therapy: According to the results of clinical trials in lymphoproliferative disorders, target therapy with monoclonal antibodies combined with conventional chemotherapy showed an improvement in response rates as well as a significant increase in progression-free and event-free survival.

The results of various international trials, obtained in Chronic Lymphocytic Leukaemia (CLL), in aggressive and indolent Lymphomas using CD20 or CD52 antibodies combined with conventional chemotherapy, will be presented.