post-splenectomy was 3.5±4.3% which is higher than the values in normal subjects (below 2%) and much lower than those found in SCD patients after total splenectomy (more than 20%). These results imply significant preservation of splenic function in our patients. No recurrence of ASS occurred after the operation. Admissions to hospital and transfusion have been reduced and the quality of life of these children and their parents has improved. There were also increases in hemoglobin levels and platelet counts, and these findings may be explained by the fact that the children with the largest spleens and recurrent ASS also have hypersplenism. Only one case of severe infection occurred. We believe that partial splenectomy is a good option for the treatment of ASS in SCD.

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Rituximab chimeric anti-CD20 monoclonal antibody treatment for refractory hemolytic anemia in patients with lymphoproliferative disorders

We administered rituximab monoclonal antibody to five patients suffering from a lymphoproliferative CD20-positive disease associated, at diagnosis or after starting treatment, with autoimmune hemolytic anemia (AIHA). After treatment with rituximab we observed an improvement of AIHA in all cases, and, in one case, improvement of the autoimmune thrombocytopenia associated with the AIHA. There were no relevant side effects.

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About 7-10% of lymphoproliferative disorders (LD) can be complicated by an autoimmune hemolytic anemia (AIHA). In some LDs, this condition is related to the production, by the neoplastic clone, of an antibody reactive against autologous red cell antigens, and is usually refractory to steroids and immunosuppressive agents.[1-3] Otherwise, the complication can be due to an imbalance in immune regulation. Often, an unknown, indolent LD can present with AIHA; alternatively, AIHA can precede a LD by a long time.

In this paper we present 5 cases of LD complicated by AIHA, that we treated successfully with an anti-CD20 monoclonal antibody (Rituximab-Mabthera®, Roche, Milan, Italy) resulting in a clinical improvement of both the AIHA and of the LD.

We treated 5 patients affected by LD with AIHA (Table 1): 2 had large-B cell non-Hodgkin's lymphoma (LBC-NHL); 2 had B-chronic lymphocytic leukemia (B-CLL) and 1 had B-prolymphocytic leukemia (B-PLL). At LD diagnosis all patients showed ≥ 27% of $CD20^+$ cells infiltration at cytofluorimetric analysis of bone marrow; in all cases, a warm autoantibody was detected. In 2 cases AIHA was the first sign of a LD, while in the other 3 patients it developed during the treatment of the LD. The diagnosis of AIHA was made as follows: decrease of the hemoglobin (Hb) levels, increase of serum lactate dehydrogenase (LDH) levels, increases of indirect and direct bilirubin levels and positivity of the direct and/or indirect antiglobulin test (DAT/IAT). Two patients had a low platelet count ($<100\times10^9$/L) before the rituximab treatment. An antiplatelet antibody was found in one of these two patients; in the other patient the serum test resulted negative. None of our patients had a monoclonal antibody related to production by the neoplastic clone. All patients received first line treatment for LD. Three were treated with CHOP (vincristine, cyclophosphamide and doxorubicin), 1 patient with MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, bleomicin), and 1 patient with Codox-M (cyclophosphamide, vincristine, doxorubicin, cytarabine, methotrexate). The median time from chemotherapy to rituximab administration was 3 months (range 1-36).

Since none of the patients achieved a stable improvement of AIHA, all patients started immunotherapy with rituximab. The schedule of administration of rituximab was four courses of 375 mg/m^2/weekly. Response to treatment was evaluated by the improvement of the parameters of hemolysis, such as decrease in bilirubin, LDH, Hb levels, and negativization or improvement of DAT/IAT (Table 2). All patients showed a recovery from AIHA, already after the second administration of rituximab; in 3 cases AIHA recovery was associated with a complete remission (CR) of LD.

Only one patient suffered from an infusion-related reaction to rituximab, consisting in chills and fever. As of June 2002, 3 patients are still alive in 1st CR (median overall survival 8 months; range 8-20) and have not so far shown any relapse of the LD or AIHA.

Another patient, whose AIHA improved, had persistent

Table 1. Features of patients treated with rituximab for AHIA.

Case	Sex/age	LD	Treatment	CR of LD	AT	Rituximab	Follow-up
1	F/44	B-CLL	CHOP	Yes	IgG/C3d	recovery	CR after 20 months
2	F/56	NHL	MACOP-B Codox-M	Yes	IgG	recovery	Relapse after 5 months
3	M/45	NHL	Codox-M IVAC	No	IgG/C3d	recovery	Dead after 3 months
4	M/57	B-CLL	CHOP	Yes	IgG	recovery	CR after 8 months
5	F/66	B-PLL	CHOP, fludarabine	Yes	IgG	recovery	CR after 10 months

B-CLL: chronic lymphocytic leukemia; NHL: large B-cell non-Hodgkin's lymphoma; B-PLL: prolymphocytic leukemia; LD: lymphoproliferative disorders; AT: antiglobulin test; CR: complete remission.

Table 2. Hemolysis parameters levels before and after rituximab treatment.

Case	Hb levels (g/dL)		DAT		IAT		LDH (UI/L)		Bilirubin (mg/dL)		Plts×10⁹/L	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	4.7	9.8	4+	2+	1+	neg	830	500	3.9	1.4	167	179
2	8.4	12.4	3+	2+	1+	neg	747	553	1.8	0.9	150	135
3	7.7	9.7	1+	neg	neg	neg	1100	220	2.2	1.6	33	92
4	9.8	13.5	1+	neg	neg	neg	469	353	1.8	1.5	97	136
5	6.5	11.1	3+	+	1+	neg	838	451	1.6	0.9	178	235

Hb: hemoglobin; DAT: direct antiglobulin test; IAT: indirect antiglobulin test; LDH: serum lactate dehydrogenase; Plts: platelets.

splenic LD and underwent autologous peripheral blood stem cell transplantation[5] months after rituximab treatment; following evidence of a relapse in the hemolytic complication, the patient was finally splenectomized 2 months after the transplant. The other patient who did not achieve remission from LD died 3 months after the rituximab treatment because of a fungal pulmonary infection, without signs of AIHA relapse. The 3 patients in CR did not show serious infectious complications during the follow-up.

Only a few papers report on the use of anti-CD20 monoclonal antibody in the treatment of autoimmune diseases associated with LD:[4-6] this option is obviously based on the detection of a bone marrow infiltration by CD20-positive cells.

We observed good results in recovery from acute hemolysis: the clinical conditions and laboratory parameters all our patients improved, and the treatment was generally well tolerated. In our experience, clinical and laboratory findings show that immunotherapy can be useful in the treatment of AIHA associated with LD. Furthermore, recent studies show the effectiveness of rituximab on autoimmune pathologies not associated with a LD,[7-10] such as Idiopathic thrombocytopenic purpura and Evans' syndrome.

Treatment with rituximab results in a depletion of normal and malignant B-cells which persists for 6 to 9 months. This B-cell depletion does not lead to either a decrease in immunoglobulin levels or an increase in the number of infectious complications. However, the effect of rituximab treatment on immune responsiveness is unknown.[11] Only one of our patients, who did not reach CR, had an infectious complication.

In conclusion, anti-CD20 monoclonal antibody in patients suffering from a LD is an effective, well tolerated therapeutic option for the management of refractory AIHA. Furthermore, the efficacy of rituximab on the LD allows the treatment of patients to continue even when their clinical conditions are not compatible with chemotherapy.

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Immunophenotypic analysis in 119 patients with acute myeloid leukemia following a previous malignancy: a comparison with the immunophenotype of 231 *de novo* cases

Data regarding the immunophenotypic pattern of 119 cases of acute myeloid leukemia (AML) following a previous malignancy were matched with those of 231 patients with *de novo* AML in order to identify differences between the 2 groups. We documented the presence of immunophenotypic markers (CD4, CD16, HLA-DR, CD33, CD117) preferentially expressed in *de novo* AML with respect to AML following a previous malignancy. On the other hand, we demonstrated that there are no differences in antigenic profile between AML following a previous malignancy treated with surgery alone and AML following a previous malignancy treated with chemo- and/or radiotherapy.

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The prognostic relevance of immunophenotype in acute myeloid leukemia (AML) is still controversial[1-10] and, to date, no studies have been performed in patients with secondary AML.

In the present study, we analyzed the immunophenotypic pattern of AML following a previous malignancy in order to investigate: the possible prognostic role of immunophenotype in AML following a previous malignancy, to identify immunophenotypic differences between *de novo* AML and AML following a previous malignancy and to compare the immunophenotype of patients with AML following a previous malignancy treated with chemo- and/or radiotherapy versus the immunophenotype of AML following a previous malignancy treated with surgery alone. The study population comprised 350 AML patients observed in 5 Divisions of Hematology from July 1992 to June 2000: 119 of the cases of AML followed a previous malignancy whereas 231 of the patients had *de novo* AML. For each patient clinical and biological characteristics were analyzed: age, sex, WBC count at diagnosis, FAB category, platelet count, hemoglobin level, karyotype, induction treatment, achievement rate and duration of complete remission (CR), and overall survival. Moreover, in the 119 cases of AML following a previous malignancy patients further data were collected: type and date of onset of the previous malignancy, treatment (chemotherapy, radiotherapy, surgery) and outcome of the previous malignancy, and latency between the previous malignancy and AML. Patients with a previous myelodysplastic syndrome not secondary to previous malignancy were excluded from this study. Cytogenetic risk groups were defined as reported elsewhere.[11]

The immunophenotypic pattern of AML following a previous malignancy was compared with that of *de novo* cases of AML according to age and FAB category (1:2 ratio). The following monoclonal antibodies were used as the first-line panel: CD2, CD3, CD4, CD5, CD7, CD9, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD22, CD33, CD34, CD38, CD41, CD45, CD56, CD61, CD117, HLA-DR, MPO. In addition, cells were labeled with antibodies directed against My8. Clinical and biological features of the 119 cases of AML following a previous malignancy are summarized in Table 1. The median latency between the two malignancies was 48 months (range 8-480). All patients were treated for AML, according to the different trials currently in use in the Institutions participating in the study. CR was achieved in 57 patients (48%), 16 patients were resistant (13%), while 38 patients (32%) died during induction chemotherapy. Eight patients (7%) achieved a partial remission (PR).

The expression of informative antigens in the two groups of 350 assessable adult AML patients is presented in Table 2. Patterns of antigen expression in *de novo* AML and AML following a previous malignancy differed significantly: in particular, CD4

Table 1. Clinical and biological features of patients with sAML.

Patients, no.	119
Age, mean (range)	58 (15-89)
Sex (M/F)	46/73
Primary malignancy:	
Breast	33
Hodgkin's disease	15
Lymphomas	15
Bowel	9
Lung	5
Kidney	5
Gut	4
Uterus	4
Ovary	4
Pharynx-larynx	4
Myelofibrosis	4
Bladder	3
Central nervous system	2
Multiple myeloma	2
Myeloproliferative chronic disease	2
Melanoma	2
Prostate	2
Skin	1
Thyroid	1
Vagina	1
Esophagus	1
Treatment of primary malignancy:	
Surgery	37
Chemotherapy	39
Radiotherapy	15
Combined chemotherapy and radiotherapy	28
FAB:	
M_0	9
M_1	20
M_2	27
M_3	15
M_4	21
M_5	21
M_6	3
M_7	3
Karyotype (on 67 patients):	
Good prognosis	5
Intermediate prognosis	45
Unfavorable prognosis	17
Response to chemotherapy	
Complete remission	57
No response	16
Death in induction	38
Partial remission	8