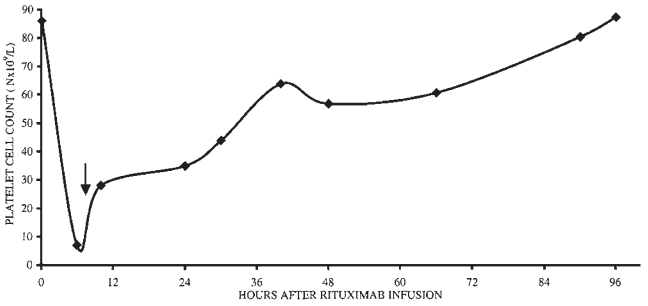

Figure 1 Monitoring of platelet count during 96 h after the onset of the first rituximab infusion. At baseline, the patient's platelet count was 86×10^9/l. Rituximab infusion was started at time 0, lasted approximately 3 h and was followed by a drastic fall in platelet count by the 6 h. The patient received a platelet transfusion and prednisolone 100 mg i.v. (indicated by an arrow in the figure), after which the platelet count was 28×10^6/l. Subsequently, the platelet count rose spontaneously and returned to baseline levels by 96 h.

els after 96 h. Ultrasonogram investigation of the spleen did not reveal significant size variation compared to baseline. Ten days after the first rituximab infusion, the patient received rituximab in a fractioned infusion schedule:[6] on day 1, 50 mg rituximab was administered over 5–7 h; on day 2, 100 mg over the same period of time; on day 3, the remainder of the 375 mg/m^2 dose. No changes in platelet cell count or LDH were observed during the 3 days of therapy, whereas liver enzymes displayed a mild increase.

The implications of this case report are three-fold. Firstly, rituximab infusion may be associated with a severe toxic syndrome, overall compatible with the previously described cytokine release syndrome, despite the absence of peripheral blood invasion by the tumor. Secondly, monitoring of platelet counts should be mandatory in all patients at risk for rituximab-induced thrombocytopenia, including patients with massively invaded spleens. Thirdly, fractioned infusion schedule may offer advantages in patients at risk of cytokine release syndrome. It is conceivable that, in our patient, tumor lysis in the context of massive splenomegaly may have sustained the toxic syndrome, which is otherwise caused by leukemic cell lysis.[6–8] As in leukemic patients the severity of the cytokine release syndrome correlates with the degree of peripheral blood invasion,[6–8] future studies need to address whether the toxic effects of rituximab in non-leukemic patients are directly related to the size of organomegaly.

C Rigamonti
C Volta
S Colombi
L Forti
F Savinelli
G Gaidano
E Bartoli

Division of Internal Medicine, Department of Medical Sciences, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy

References

1 Maloney DG, Grillo-Lopez AJ, White CA, Bodkin DJ, Schilder RJ, Neidhart JA, Janakiraman N, Foon KA, Liles TM, Dallaire BK, Wey K, Royston I, Davis T, Levy R. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* 1997; **90**: 2188–2195.
2 Maloney DG, Grillo-Lopez AJ, Bodkin DJ, White CA, Liles T-M, Royston I, Varns C, Rosemberg J, Levy R. IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed low-grade non-Hodgkin's lymphoma. *J Clin Oncol* 1997; **16**: 3266–3274.
3 McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 1998; **16**: 2825–2833.
4 Feuring-Buske M, Buske C, Unterhalt M, Hiddemann W. Recent advances in antigen-targeted therapy in non-Hodgkin's lymphoma. *Ann Hematol* 2000; **79**: 167–174.
5 Hainsworth JD, Burris III HA, Morrisey LH, Litchy S, Scullin DC Jr, Bearden JD III, Richards P, Greco FA. Rituximab monoclonal antibody as initial systemic therapy for patients with low-grade non-Hodgkin's lymphoma. *Blood* 2000; **95**: 3052–3056.
6 Winkler U, Jensen M, Manzke O, Shultz H, Diehl V, Engert A. Cytokine-release syndrome in patients with B cell chronic lymphocytic leukemia and high lymphocitic counts after treatment with an anti-CD20 monoclonal antibody (rituximab, IDEC-C2B8). *Blood* 1999; **94**: 2217–2224.
7 Byrd JC, Waselenko JK, Maneatis TJ, Murphy T, Ward FT, Monahan BR, Sipe MA, Donegan S, White CA. Rituximab therapy in hematologic malignancy patients with circulating blood tumor cells: association with increased infusion-related side effects and rapid blood tumor clearance. *J Clin Oncol* 1999; **17**: 791–795.
8 Lim LC, Koh LP, Tan P. Fatal cytokine release syndrome with chimeric anti-CD20 monoclonal antibody rituximab in a 71-year-old patient with lymphocytic leukemia. *J Clin Oncol* 1999; **17**: 1962–1963.

Remission of severe cold agglutinin disease after Rituximab therapy

TO THE EDITOR

Rituximab (Mabthera; Roche, Brussels, Belgium) has demonstrated activity in the treatment of non-Hodgkin's lymphoma (NHL) of B cell origin.[1] This antibody, which binds specifically to the CD20 antigen, a pan B cell marker, can deplete B cells through complement- and antibody-dependent cellular cytotoxicity. Rituximab is presumably active in Waldenström's macroglobulinemia (WM) because of the elimination of either CD20$^+$ clonotypic precursor B cells, or CD20$^+$ plasma cells, which have both been detected in most WM patients.[2] The same rationale could apply to other immunoglobulin-mediated diseases of B lymphocytes, such as cold agglutinin disease (CAD) or cryoglobulinemia, which could also theoretically benefit from CD20 targeted therapy. We hereby describe a patient with refractory CAD who went into remission after therapy with Rituximab.

A 67-year-old patient presented in April 1997 for acrocyanosis, weakness and dyspnoea. Her medical history included the diagnosis of an indolent NHL of the bone marrow (BM) in 1993, with secondary CAD. She had received chlorambucil (CLB) daily (5 mg/d) from January 1993 until April 1996 with resolution of signs of hemolysis. There were no enlarged lymph nodes. There was no hepatosplenomegaly. Laboratory studies showed a hemoglobin of 7.6 g/dl, a hematocrit of 23%, and a reticulocyte count of 66 000/μl. The white blood cell count was 1770/μl with 51% neutrophils, 32% lymphocytes, and no abnormal forms. The platelet count was 37 000/μl. LDH were at 610 UI/l and total bilirubin was 1.7 mg/dl. Haptoglobin level was $<$12 mg/dl. The direct antiglobulin test was positive with IgM and complement. At elution, a monoclonal IgM with anti-I cold-agglutinin activity was found. BM analysis showed a diffuse and nodular lympho-plasmocytoid infiltrate (52% of total cellularity) with focal reticulinic fibrosis, together with erythroid hyperplasia and discrete dyserythropoiesis. Megakaryocytes were at the lower normal limit. Clonal rearrangement of the immunoglobulin heavy chain gene was found.

Correspondence: E Van Den Neste, Service d'Hématologie, Cliniques Universitaires Saint-Luc, 10 av. Hippocrate, 1200 Brussels, Belgium; Fax: 32 2 7648959
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The karyotype was normal on 10 mitoses. Whole-body CT scan was normal.

Because of pancytopenia, corticosteroid therapy was preferred to resuming CLB. She received sequential dexamethasone (20 mg/d for 4 days, 3 cycles per month during 2 months, followed by 1 cycle per month for 3 months), which was relayed by oral methylprednisolone. The transfusion need remained at 2 units of RBC every 3 weeks. In October 1997, there was a flare up of hemolysis, which led to the initiation of plasmapheresis together with 3 weekly doses of vincristine (1 mg each). A slight improvement was observed, as evidenced by a rise in haptoglobin and hemoglobin levels, without influence on the transfusion rate. Cyclophosphamide (two doses of 1 g each, at 4 weeks interval) did not improve the hemolysis. A BM examination was performed in August 1998 and proved to be similar to the initial one. Immunophenotyping showed monoclonal kappa light chain B lymphocytes expressing the CD20 antigen. High-dose corticosteroid therapy (methylprednisolone 1 g/6 week) was again attempted, along with plasmapheresis and vincristine, without benefit. Reticulocyte counts above 150 000/μl were regularly observed throughout the evolution. In November 1998, the patient's hemolytic process was still active as shown by LDH and total bilirubin levels of 843 UI/l and 1.6 mg/dl, respectively, and haptoglobin <12 mg/dl. The IgM level was 409 mg/dl. The patient was included in a protocol comprising induction with Rituximab and maintenance with interferon-α2a (INFα, Roferon; Roche) for the treatment of indolent NHL. She received 4 weekly doses of Rituximab (375 mg/m^2 each), with good tolerance. No steroids were given. INFα was initiated 2 weeks after the last dose of Rituximab at the dose of 4.5×10^6 units, three times weekly, and continued for 16 weeks. Two months after starting Rituximab, LDH and total bilirubin had fallen to 412 UI/lL and 0.9 mg/dl, respectively. From then, the patient remained free of hemolysis for 7 months. Haptoglobin level and IgM component were 174 mg/dl and 59 mg/dl, respectively, in June 1999, when the patient was hospitalized for an epidural tumor (L5-S1) complicated by neurological compression. BM examination showed a normal cellularity, discrete reticulinic fibrosis, and no lymphomatous infiltration. There was no B cell monoclonality by flow cytometry analysis or Southern blotting. The tumor was thought to be lymphomatous, but no biopsy was performed because of thrombocytopenia. Radiotherapy was delivered, leading to a good response. In August 1999, hemolysis recurred severely and continued unabated despite corticosteroids and danazol. The patient died in February 2000 from a stroke.

This patient had an indolent clonal lymphoproliferative disease limited to the BM, a seric monoclonal IgM component with cold agglutinin activity, and severe hemolysis poorly responsive or refractory to prevention to cold exposure, corticosteroids, plasmapheresis, vincristine and high-dose cyclophosphamide. A lasting remission of hemolysis was achieved after Rituximab. Since hemolysis improved during Rituximab therapy, and went into remission after only six doses of INFα, it is unlikely that INFα played a role in inducing remission, although it may have contributed to its maintenance through an effect on the underlying NHL. Yet, the documented efficacy of INFα in CAD is very limited.[3]

A similar effect of Rituximab, in combination with corticosteroids and cyclophosphamide, has been reported in a patient with CAD secondary to NHL.[4] Significant improvement has also been shown in a patient with refractory type II cryoglobulinemia after Rituximab therapy.[5] Finally, B cell depletion with Rituximab also proved to be effective in IgM-related polyneuropathies, as shown by clinical improvement and decrease in serum autoantibodies titers.[6] These observations encourage studies testing Rituximab in diseases characterized by the production of autoantibodies. In patients in whom a lymphoproliferative disorder is associated, the addition of interferons might prove useful since cytokines may modulate some of the immune effectors, such as antibody-dependent cellular toxicity or CD20 expression, involved in the cytotoxic effects of monoclonal antibodies.[7,8] In this situation, the respective schedules of administration of these two drugs remain to be determined.

N Layios
E Van Den Neste
E Jost
V Deneys
JM Scheiff
A Ferrant

Services d'Hématologie et de Biologie Hématologique, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium

References

1 Maloney DG, Grillo-Lopez AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, Janakiraman N, Foo KA, Liles TM, Dallaire BK, Wey K, Royston I, Davis T, Levy R. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. *Blood* 1997; **90**: 2188–2195.
2 Byrd JC, White CA, Link B, Lucas MS, Velasquez WS, Rosenberg J, Grillo-Lopez AJ. Rituximab therapy in Waldenstrom's macroglobulinemia: preliminary evidence of clinical activity. *Ann Oncol* 1999; **10**: 1525–1527.
3 O'Connor BM, Clifford JM, Lawrence WD, Logue GL. Alpha-interferon for severe cold agglutinin disease. *Ann Intern Med* 1989; **111**: 255–256.
4 Lee EJ, Kueck B. Rituxan in the treatment of cold agglutinin disease. *Blood* 1998; **92**: 3490–3491.
5 Zaja F, Russo D, Fuga G, Patriarca F, Ermacora A, Baccarani M. Rituximab for the treatment of type II mixed cryoglobulinemia. *Haematologica* 1999; **84**: 1157–1158.
6 Levine TD, Pestronk A. IgM antibody-related polyneuropathies: B-cell depletion chemotherapy using Rituximab. *Neurology* 1999; **52**: 1701–1704.
7 Venugopal P, Sivaraman S, Huang X, Chopra HK, O'Brien T. Jajeh A, Preisler HD. Upregulation of CD20 expression in chronic lymphocytic leukemia (CLL) cells by *in vitro* exposure to cytokines. *Blood* 1998; **92**: 247a (Abstr. 1009).
8 Bungard S, Flieger D, Schweitzer S, Sauerbruch T, Spengler U. The combination of interleukin-2 and interferon alpha effectively augments the antibody-dependent cellular cytotoxicity of monoclonal antibodies 17–1A and BR55–2 against the colorectal carcinoma cell line HT29. *Cancer Immunol Immunother* 1998; **46**: 213–20.

Age cohort subgroups in adult acute myeloid leukaemia studies – the population perspective

TO THE EDITOR

Survival in acute leukaemia is age-dependent and unintentional age biases in trials/studies may be a major cause of inconsistency when comparing outcome between studies. Assessing clinical outcome using a population-based data set collected over a prolonged time period linked to cytogenetics has revealed the full extent of the heterogeneity based on age, suggesting a rethink of study design in leukaemia trials is required.

Acute myeloid leukaemia (AML), in the adult age group (>15 years), is a heterogeneous disease group with differing chromosomal abnormalities associated with the different biological subtypes of the disease, the incidence of subtypes varying over different age groups. Response to therapy varies between age groups, in part due to the different nature of the disease. Few studies in leukaemia have been conducted on unselected cohorts of patients and trials continue to be

Correspondence: SJ Proctor; Fax: +44 (0)191 222 7632
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