B-cell depletion with rituximab as treatment for immune hemolytic anemia and chronic thrombocytopenia

Francesco Zaja,* Isabella Iacona,° Paola Masolini,* Domenico Russo,* Alessandra Sperotto,* Simonetta Prosdocimo,* Francesca Patriarca,* Salvatore de Vita,@ Mario Regazzi,° Michele Baccarani,# Renato Fanin*

*Clinica Ematologica, Policlinico Universitario, Udine, °Dipartimento di Farmacologia, IRCCS Policlinico S. Matteo, Pavia; #Istituto di Ematologia ed Oncologia Medica L. e A. Seragnoli, Bologna; @Clinica Reumatologica, DPMSC, Policlinico Universitario, Udine, Italy

Background and Objectives. Rituximab reacts specifically with the CD20 antigen and induces B-cell depletion. This could interfere with the production of autoantibodies in some immune diseases. The objective of this study was to assess the effects of rituximab in autoimmune hemolytic anemia and thrombocytopenia.

Design and Methods. Seven patients (one with cold agglutinin disease, two with warm antibody autoimmune hemolytic anemia, four with chronic idiopathic thrombocytopenic purpura) previously refractory to conventional treatments were treated with weekly infusions of rituximab, 375 mg/m^2, for 4 weeks. Only treatment with steroids, if strictly necessary, was allowed during the period of rituximab administration, but only patients who reached steroid suspension were considered responders. The pharmacokinetics of rituximab were quantified during therapy and the follow-up period.

Results. All patients had marked, even if temporary, B-cell depletion. Three patients, 1 with cold agglutinin disease (CAD) and 2 with chronic idiopathic thrombocytopenic purpura (ITP), had a complete hematologic response. In the patient with cold agglutinin disease a decrease in the agglutinin titer was observed. The hematologic improvement was prompt, appearing by the second or third infusion of rituximab. The response duration was CAD 96+, ITP 17+ and 13+ weeks in these 3 patients. Treatment tolerance was satisfactory and no infections or other late events were registered. Serum rituximab concentrations appeared to be similar to those calculated in a historical control group of patients with follicular non-Hodgkin's lymphoma who received rituximab as consolidation of response after first-line CHOP chemotherapy.

original paper

haematologica 2002; 87:189-195
http://www.haematologica.it/2002_02/189.htm

Correspondence: Francesco Zaja, MD, Division of Hematology, University Hospital, p.zza S. Maria della Misericordia, 33100 Udine, Italy. Phone: international +39.432.559662. Fax: international +39.432.559661. E-mail: ematologia@drmm.uniud.it

Interpretation and Conclusions. Rituximab appeared to be active and safe in some patients with refractory autoimmune hemolytic anemia and thrombocytopenia. These results, along with data from literature, suggest that this agent may have a therapeutic role in autoimmune diseases.

Key words: rituximab, B-cell depletion, autoimmune hemolytic anemia, chronic idiopathic thrombocytopenic purpura.

Several diseases or syndromes are associated with the production of polyclonal or monoclonal immunoglobulins that have abnormal physical properties and, in many cases, react with self antigens carried by normal cells and tissues. Since immunoglobulins are produced by B-lymphocytes and plasmacells, the use of a drug which is specific for the B-cell compartment may help to control the secretion of abnormal antibodies. Autoimmune hemolytic anemia (AHA) and idiopathic thrombocytopenia (ITP) are hematologic diseases associated with the production of antibodies against red cells and platelets, respectively.

Rituximab is a human-mouse chimeric monoclonal antibody (IgG 1κ)[1] specific for the CD20 antigen, found on the surface of B-lymphocytes where it acts through complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity. CD20 appears to be a suitable target for immunotherapy because it is not expressed on hemopoietic stem cells or other body tissues, does not circulate in the plasma as free protein, is not shed from the surface after binding of the anti-CD20 monoclonal antibody and does not internalize upon antibody binding. This agent showed good

activity in the treatment of relapsed, low-grade B-cell CD20+ non-Hodgkin's lymphoma (NHL).[2,3] In particular, major activity was observed in patients with follicular NHL, in which even a molecular status of remission could be reached. Toxicity was generally mild and consisted primarily of infusion-related events. Initial studies on animals and, subsequently, on patients treated with rituximab, showed the development of marked (even if transitory) B-cell depletion from peripheral blood, bone marrow and lymph nodes, while only mild modifications of immunoglobulin and complement serum levels were found.

The good therapeutic index of this drug recently led to the use this agent, besides in neoplastic diseases, in autoimmune disorders, with the aim of acting on B-cells and interfering with the production of autoantibodies. Preliminary results appeared encouraging since significant remissions were reached in patients previously refractory to conventional treatments.

We report the results of our experience with rituximab in seven patients with hematologic autoimmune diseases, giving the update of a case of cold agglutinin disease (CAD) already reported[4] and referring the results observed in two newer cases with warm antibody AHA and in four cases with chronic ITP. We also report the serum concentrations of rituximab in these patients, quantified during therapy and the follow-up period. This quantification was performed to characterize drug availability in a population of patients with a reduced number of total B-cells. Data available on this issue are lacking, despite several studies having been conducted in lymphoma patients.

Design and methods

Patients

In order to be included in this study, patients had to be 18 or more years old, HIV negative, with active and symptomatic AHA (warm antibody or cold agglutinin) or ITP resistant to full dose conventional treatments. The patients' main clinical and laboratory features and treatments prior to rituximab are summarized in Table 1. One of the patients with warm antibody AHA also had a B-cell chronic lymphocytic leukemia (CLL) stage 0 Rai. Informed written consent to the study was obtained from the patients.

Treatment with rituximab

Rituximab (Mabthera, Roche S.p.a., Milan, Italy) was given at a dose of 375 mg/m^2 iv on days +1, +8, +15, and +22. The initial infusion rate was 50 mg/h, with a subsequent infusion-rate increase up to 300 mg/h if no toxicity was seen. Patients received oral acetaminophen 500 mg and iv chlorphenamine 10 mg as premedication therapy. No other cytotoxic or immunosuppressive drugs were given in association with rituximab. Only treatment with steroids, if strictly necessary, was allowed during the period of rituximab administration, but only patients who reached steroid suspension were considered responders. Premature treatment withdrawal was allowed in the presence of any WHO grade IV toxicity or grade ≥ 3 neurotoxicity.

Table 1. Main clinical features of patients before treatment with rituximab.

Disease	*Pts.*	*Sex/age*	*Previous treatments*	*Months from dx*	*Main laboratory features before rituximab*
CAD	C.S.	M/72	PDN, AZA	7	Hb: 73 g/L DAT: positive Cold agglutinin titer: 1:512 LDH: 852 U/L Haptoglobin: < 80 mg/L
AHA/CLL	G.G.	M/78	PDN, AZA, CTX	60	Hb: 104 g/L DAT: positive LDH: 620 U/L Haptoglobin: 7 mg/L
AHA	M.P.	M/53	PDN, AZA	5	Hb: 61 g/L DAT: positive LDH: 872 U/L Haptoglobin: 5 mg/L
ITP	V.F.	F/65	PDN, IG	8	PLT: 10×10^9/L
ITP	C.D.	F/65	PDN	100	PLT: 18×10^9/L
ITP	T.M.	F/42	PDN, splenectomy	89	PLT: 26×10^9/L
ITP	B.A.	F/56	PDN/AZA	240	PLT: 23×10^9/L

Response criteria for AHA

A complete response (CR) was defined as a hemoglobin level ≥ 120 g/L, absence of clinical and laboratory signs of hemolysis (i.e., normalization of the reticulocyte count and of the level of lactate dehydrogenase (LDH), bilirubin, and haptoglobin), and suspension of steroid therapy. A partial response (PR) was defined as a hemoglobin level > 100 g/L, improvement of clinical and laboratory signs of hemolysis, and suspension of steroid therapy. A minor response (MR) was considered to have occurred when there was a 30 to 50% improvement of clinical and laboratory signs of hemolysis, and suspension

of steroid therapy. Patients with less than a MR were considered to be non-responders (NR).

Response criteria for ITP

A complete response was defined as a platelet level ≥ 100× 10^9/L, suspension of steroid therapy, whereas a partial response was defined as a platelet level ≥ 50×10^9/L and suspension of steroid therapy. A minor response was a platelet level ≥ 30×10^9/L with suspension of steroid therapy. Patients with less than a MR were considered non-responders (NR).

Immunologic assessment

To evaluate the main immunologic modifications after treatment with rituximab the following analyses were performed in all the patients at baseline and then monthly up to reconstitution of the baseline level: blood cell count and differential, immunophenotypic analysis of the lymphoid markers CD3, CD4, CD8, CD19, and CD20, and serum immunoglobulin levels of IgG, IgA, and IgM. The immunophenotype was evaluated in peripheral blood samples using standard procedures. Assays of cold agglutinin and the Coombs' test were carried out in patients with AHA whereas the presence of anti-platelets antibodies was checked in patients with ITP.

Pharmacokinetics

Serum rituximab concentrations in all patients were determined using a previously validated enzyme-linked immunoassay (ELISA).[5] Briefly, diluted serum samples were allowed to react with purified polyclonal anti-rituximab antibody coated on a microtiter plate and with anti-human IgG labeled with horseradish peroxidase. After incubation and washings, substrate solution was added and absorbance was read at 492 nm. Rituximab concentrations in samples were determined by interpolation from a standard curve prepared diluting known amounts of rituximab into normal human serum. Results were expressed as μg/mL. The sensitivity of the method was 2 μg/mL. Quality control samples at different rituximab concentrations were analyzed in each analytical run and showed a coefficient of variation for precision and accuracy of <15% (acceptance criterion). Blood samples for rituximab determination were taken before and immediately after each infusion (day +1, +8, +15 and +22). Additional samples were taken at weekly intervals for five weeks after the last infusion. Samples from all time points were stored at –20°C until analysis. Rituximab concentration-time data were analyzed using a statistical pharmacokinetic population software (P-Pharm, version 3, Simed, Creteil, France). For each patient, the infusions were considered as one treatment course and analyzed for the pharmacokinetic study as one group.

Table 2. Characteristics of response in a patient with CAD and two patients with ITP.

Disease	*Patients*	*Response*	*Weeks to initial response*	*Weeks to peak response*	*Response duration (mos)*
CAD	C.S.	CR	3	28	24+
ITP	T.M.	CR	3	12	4+
ITP	B.A.	CR	2	8	3+

Toxicity

Rituximab-related toxicity was assessed during the period of treatment (acute toxicity; from baseline to week 6) and during follow-up (delayed toxicity; from week 6 to week 24). Clinical and laboratory side effects were classified according to the WHO scale.

Results

Treatment program

All patients completed the therapeutic program as planned.

AHA

As previously reported,[4] the patient with CAD reached CR after treatment with rituximab. Response to treatment was rapid since hemoglobin level started improving at week +3 after the beginning of therapy and normalized at week +28. At the same time a reduction of cold agglutinin titer to a very low level was documented (Figure 1A). This allowed steroid treatment to be tapered down and eventually suspended at week +16. At present, 96 weeks after beginning rituximab therapy, the patient is well and in CR.

Before treatment with rituximab, the two patients with warm antibody AHA required continuous full dosage steroid therapy to control their hemolytic anemia, being resistant to azathioprine and/or cyclophosphamide. They both had laboratory signs of active hemolysis with anemia, reduction of haptoglobin level and raised reticulocyte counts, LDH and bilirubin levels (Table 1). Rituximab was given together with prednisone 1 mg/kg/daily. These patients were considered non-responders since, when steroid reduction was started after treatment with rituximab, all their laboratory signs of hemolysis worsened and they required restoration of the full prednisone dosage.

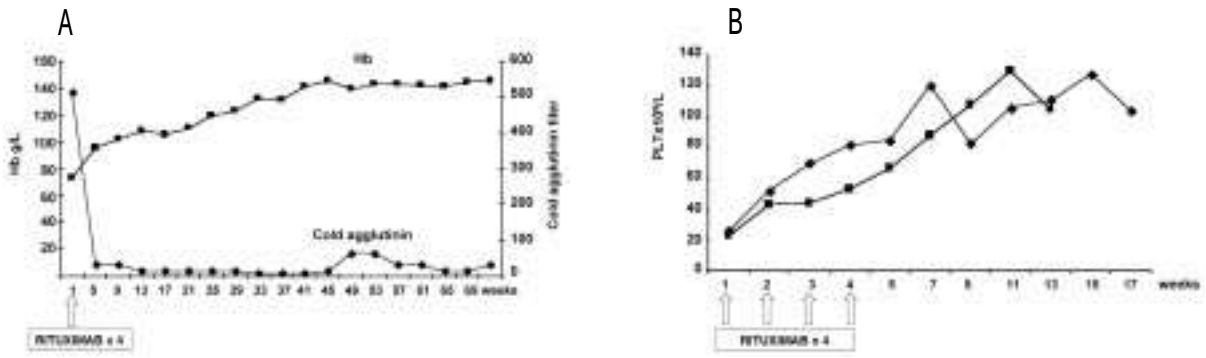

Figure 1A. Hemoglobin level (g/L) and cold agglutinin titer in a patient with CAD before and after treatment with rituximab. Figure 1B. Improvement of platelet counts in two patients with ITP.

ITP

All patients with ITP had chronic disease, requiring intermittent or continuous treatment with steroids to maintain a platelet count above 10×10^9/L. Only one had had a splenectomy before rituximab; the other patients had not been splenectomized because of old age or comorbidities. Two patients reached CR. The platelet improvement was evident even during the therapeutic course (at week 2) and the level of response was maximum after 12 and 7 weeks (Figure 1B). The duration of response in these two patients is 17+ and 13+ weeks. Two other patients, however, did not respond to rituximab and their platelet counts remained unchanged. Apart for older age (both 65 years old compared to the responders who were 42 and 56 years) the clinical presentation and laboratory data of these patients did not differ substantially from those of the two responders.

Immunophenotype modifications

All patients underwent a marked B-cell depletion documented by a simultaneous and concordant decrease in the numbers of $CD19^+$ and $CD20^+$ lymphocytes. The median counts of B-cells at baseline, and 5 and 12 weeks after the beginning of therapy were 0.35×10^9/L (range $0.095\text{-}7\times10^9$/L), 0.006×10^9/L (range $0\text{-}0.003\times10^9$/L) and 0.002 (range $0\text{-}0.006\times10^9$/L), respectively. No significant modifications in the level of $CD3^+$, $CD4^+$ and $CD8^+$ cells were documented, as expected.

Immunoglobulin modifications

Immunoglobulin serum level modifications depended on the type of disease and on the immunoglobulin class. In the patient with CAD the levels of IgM at weeks 1, 24, and 48 were 1 g/L, 0.6 g/L, and 0.7 g/L, respectively; at the same time the IgG levels were 4 g/L, 6.2 g/L and 7 g/L, respectively. A reduction of IgG levels from 4.8 and 6.1 g/L at week 1 to 2.5 and 4.2 g/L at week 24 were observed in the two other patients with AHA. No modifications of the immunoglobulin levels were registered in the patients with ITP.

Pharmacokinetics

As shown in Figure 2, a steady increase in pre- and post-infusion median rituximab concentrations was observed during therapy at all scheduled time points. At the start of the second infusion, the median rituximab serum concentration was 74.2 μg/mL and one week after the 4th infusion it was 176.4 μg/mL. The extent of rituximab accumulation calculated by comparing these values is 2.38. A slow, continued decline during the post-treatment period was observed with rituximab still being detectable until the 5^{th} week after the last infusion (median 43.0 μg/mL). Rituximab disposition was characterized by exponential decay, with a median elimination half-life of 468.7 hours (range 394.0-576.2) The serum rituximab concentrations in this group of patients appear to be similar to those calculated in patients with follicular non-Hodgkin's lymphoma who received rituximab as consolidation of response after first-line CHOP chemotherapy (Figure 2). The serum antibody concentration-time curve in a typical patient from the average population estimated by the pharmacokinetic program is reported in Figure 3.

Toxicity

Rituximab appeared to be tolerated quite satisfactorily and no evidence of typical infusion-related side effects, infectious complications or other late events were seen in any patient.

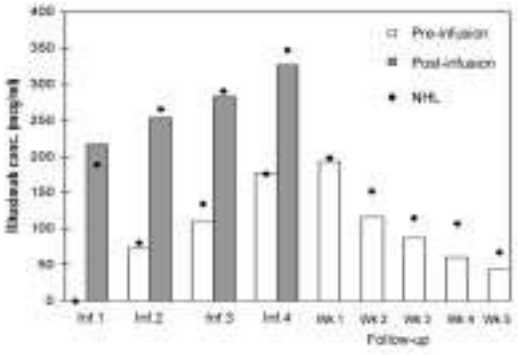

Figure 2. Comparison between median pre- and post-infusion rituximab serum concentrations obtained in 7 patients with autoimmune hemolytic anemia or thrombocytopenia and in 7 NHL patients.

Discussion

Rituximab was used for the treatment of patients with refractory AHA and ITP based on the assumption that the induction of B-cell depletion could interfere positively with the production of pathologic antibodies against red cells and platelets. In three of the seven patients treated with rituximab (1 with CAD and 2 with ITP) this allowed a very rapid improvement of hemoglobin level or platelet count up to CR (Table 2). In the patient with CAD, the efficacy of rituximab on cold agglutinin production was clearly documented. This patient, previously refractory to treatment with prednisone and azathioprine, after one course of rituximab had a complete and durable normalization of the level of hemoglobin and hemolytic signs of disease together with a reduction of the assay of cold agglutinin to nearly undetectable levels. The results in two patients with ITP were also impressive, in so much as these patients had a very long history of disease and were previously refractory to at least two conventional lines of therapy including splenectomy in one patient.

The activity of rituximab in patients with autoimmune hemolytic anemia and thrombocytopenia has also been recently reported by other groups. Most of the reports were related to a very small number of patients refractory to standard treatments. The therapeutic schedule was the same as that initially used for NHL, i.e. 375 mg/m^2 once weekly for 4 infusions. Lee *et al.*[6] treated 5 patients with CAD or warm antibody AHA. All patients had a CR and response duration ranged between 4 and 32 months. One patient was rechallenged after 5 months obtaining a second remission. Berentsen *et al.*[7] treated 5 patient with primary CAD; 1 obtained a CR and 3 had a PR. Layios *et al.*[8] and Bauduer[9] gave rituximab to a patient with an indolent NHL and CAD refractory to steroids and cytotoxic drugs achieving a lasting remission from hemolysis. Similarly, Ahrens *et al.*[10] reported a case of warm antibody AHA previously resistent to steroids, immunosuppressive and cytotoxic drugs whose hemoglobin concentration and hemolysis improved significantly after rituximab. Finally, Zecca *et al.*[11] reported the hematologic remission achieved in an 18-month old girl with both pure red cell aplasia and AHA. There are more data on patients with refractory chronic ITP. The largest study was recently reported by Stasi *et al.*[12] who gave rituximab to 25 patients previously treated with at least 2 lines of

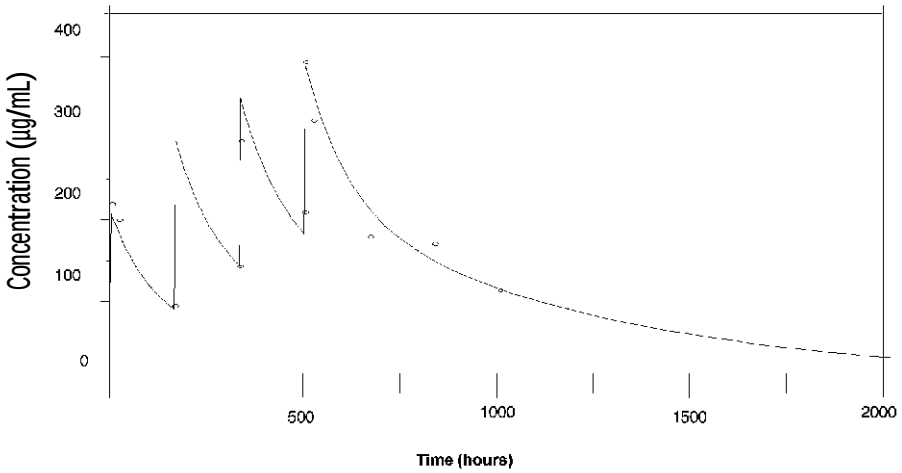

Figure 3. Rituximab serum concentration profile in a typical patient.

treatment (splenectomy in 8 patients), with a platelet count less than 20×10^9/L and/or significant bleeding. Overall response was 53% with 5 patients achieving a CR, 5 a PR and 3 a minor response. Responding patients had a rapid increase in platelet count (even after the first rituximab infusion) and the peak response was reached within 4 weeks after the end of treatment. Response duration ranged between 6 to 108+ weeks. Four patients were retreated with rituximab after relapse and a new response was observed in 2. No relationship could be found between patients' characteristics and response. Perotta *et al.*[13] treated 10 patients and recorded 5 CRs, 1 PR and a response duration which ranged from 1 to 14 months. Saleh *et al.*,[14] in a dose finding trial, observed rituximab activity in only 3/13 patients (2 CRs and 1 PR) treated at the highest dose level of 375 mg/m^2 ×4 weekly doses. The results of these and other reports are summarized in Table 3.

In our experience rituximab was very well tolerated and no significant infusion-related or late events were registered. In particular the feared increase of infectious complications because of B-cell depletion and the pre-existent immunosuppressed condition of most patients was not observed. Our data, with those in the literature, confirm the patients' good compliance to this treatment and the absence of significant side effects.

Serum rituximab availability during and after therapy was also assessed and well characterized by means of the adopted pharmacokinetic model. Drug accumulation and its extent could be a result of administration frequency, since a very long time is required for drug distribution and elimination. Comparison of these data with those obtained in a historical group of NHL patients[5] showed a very similar trend of drug disposition. Despite previous pharmacokinetic studies[17-19] having demonstrated an association between clinical response and serum rituximab accumulation both during and after treatment, in our patients no difference was observed in serum levels between responders and non-responders. However, the number of patients enrolled in the present study is too small to draw conclusions and future studies should be planned to evaluate correlations between serum rituximab concentrations and clinical parameters and/or pharmacodynamics.

The mechanism of action of rituximab in autoimmune diseases should be further investigated. Since responding patients with ITP usually start improving very rapidly (even after the first or second infusion), it has been postulated that this is unlikely to be the result of a drop in the autoantibody level and that a mechanism of Fc-receptor saturation of reticuloendothelial cells by opsonized CD20$^+$ cells may be involved to justify the initial phase of response.[12] However, in our experience, the decrease of the cold agglutinin titer was very rapid, falling from 1:512 at baseline to 1:32 and 1:16 at weeks 2 and 4, respectively, from the beginning of treatment. Similarly, in a patient treated with rituximab for refractory myasthenia gravis,[20] after an initial increase at week 2 of the antibodies against the acetyl-choline receptor, from week 4 the patient started to show a reduction of autoantibodies together with a significant improvement of clinical symptoms.

The mechanisms of resistance to rituximab in these cohorts of patients remain, at present, unknown. Since all patients develop B-cell depletion it can be assumed that other factors not strictly related to rituximab and probably secondary to T-cell deregulation could be involved.

In conclusion, rituximab appears to be a promising agent for the treatment of hematologic autoimmune diseases. Rituximab compares favorably in patients refractory to cytotoxic or immunosuppressive drugs in terms of response and tolerance. Prospective controlled trials are warranted to understand the real therapeutic impact of this agent in autoimmune diseases and to generate the new biological knowledge necessary to select both

Table 3. Rituximab for the treatment of immune hemolytic anemia and thrombocytopenia: data from literature.

References	*Disease*	*Patients*	*Treatment schedule*	*Response*	*Response duration (mos)*
Lee[6]	CAD/AHA	5	375 mg/m^2 × 4	5 CR	4-32
Berentsen[7]	CAD	5	375 mg/m^2 × 4	1 CR,3 PR	NA
Layios [8]	CAD	1	375 mg/m^2 × 4	CR	9
Bauduer [9]	CAD	1	375 mg/m^2 × 4	CR	9+
Ahrens [10]	AHA	1	375 mg/m^2 × 4	PR	6+
Zecca [11]	PRCA+AHA	1	375 mg/m^2 × 2	CR	5+
Stasi [12]	ITP	25	375 mg/m^2 × 4	5 CR,5 PR,2MR	6-108+
Perotta [13]	ITP	10	375 mg/m^2 × 4	5CR,1PR	1-14
Saleh [14]	ITP	13	375 mg/m^2 × 4	2CR,1PR	NA
Mow [15]	ITP	1	375 mg/m^2 × 4	CR	7+
Ratanatharathorn[16]	ITP(cGVHD)	1	375 mg/m^2 × 4	CR	10+

mos: months; CAD: cold agglutinin disease; AHA warm antibody autoimmune hemolytic anemia; PRCA: pure red cell aplasia; ITP: chronic idiopathic thrombocytopenic purpura; cGVHD: chronic graft-versus-host disease; CR: complete response; PR: partial response; MR: minor response; NA: not available.

the patients and the diseases which could benefit from such a treatment.

Contributions and Acknowledgments

FZ: primarily responsible for the conception and design of the study and the analysis and interpretation of data; FZ and II: writing of the manuscript; II and MR: performed and interpreted all pharmacokinetic data; PM: performed immunophenotypic analyses; DR, AS, SP, FP, RF: participated in the patients' care and contributed to the interpretation of the results; all authors gave their final approval to the study.

Disclosures

Conflict of interest: none.

Redundant publications: yes, < 50%.

References

1. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, et al. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 1994; 83:435-45.
2. Maloney DG, Grillo-Lopez AJ, White CA, Bodkin D, Schilder RJ, Neidhart JA, et al. IDEC-C2B8 (rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 1997; 90:2188-95.
3. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al. Rituximab chimeric anti-CD20 monclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16:2825-33.
4. Zaja F, Russo D, Fuga G, Michelutti T, Sperotto A, Fanin R, et al. Rituximab in a case of cold agglutinin disease. Br J Haematol 2001; 115:232-3.
5. Iacona I, Lazzarino M, Avanzini MA, Rupolo M, Arcaini L, Astori C, et al. Rituximab (IDEC-C2B8): validation of a sensitive enzyme-linked immunoassay applied to a clinical pharmacokinetic study. Ther Drug Monit 2000; 22: 295-301.
6. Lee E, Zamkoff KW, Gentile TC, Zimrin A. Rituxan in the treatment of auto-immune haemolytic anemia (AIHA). Blood 2000; 96:2560a [abstract].
7. Berentsen S, Tjonnfjord GE, Gjertsen BT. Rituxan (rituximab) therapy for chronic cold agglutinin disease. Blood 2000; 96: 3156a [abstract].
8. Layios N, Van Den Neste E, Jost E, Deneys V, Scheiff JM, Ferrant A. Remission of severe cold agglutinin disease after rituximab therapy. Leukemia 2001; 15:187-8.
9. Bauduer F. Rituximab: a very efficient therapy in cold agglutinins and refractory autoimmune haemolytic anaemia associated with CD20-positive, low-grade non-Hodgkin's lymphoma. Br J Haematol 2001; 112:1085-6.
10. Ahrens N, Kingreen D, Seltsam A, Salama A. Treatment of refractory autoimmune haemolytic anaemia with anti-CD20 (rituximab). Br J Haematol 2001; 114:244-5.
11. Zecca M, De Stefano P, Nobili B, Locatelli F. Anti-CD20 monoclonal antibody for the treatment of severe, immune-mediated, pure red cell aplasia and hemolytic anemia. Blood 2001; 97:3995-7.
12. Stasi R, Pagano A, Stipa E, Amadori S. Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 2001; 98:952-7.
13. Perotta A, Sunnemberg JS, Ratanatharathorn V. Rituxan in the treatment of chronic idiopathic thrombocytopenia purpura (ITP). Blood 1999; 94:49a [abstract].
14. Saleh MN, Gutheil J, Moore M. A pilot study of anti-CD20 MoAb rituximab in patients with refractory immune thrombocytopenic purpura (ITP). Blood 2000; 96:1086a [abstract].
15. Mow M, Hook CC. Rituximab for the treatment of refractory immune thrombocytopenic purpura. Blood 2000; 96: 3526a[abstract].
16. Ratanatharathorn V, Carson E, Reynolds C, Ayash LJ, Levine J, Yanik G, et al. Anti-CD20 chimeric monoclonal antibody treatment of refractory immune-mediated thrombocytopenia in a patient with chronic graft-versus-host disease. Ann Intern Med 2000; 133:275-9.
17. Berinstein NL, Grillo-Lopez AJ, White CA, Bence-Bruckler I, Maloney D, Czuczman M, et al. Association of serum rituximab (IDEC-C2B8) concentration and anti-tumor response in the treatment of recurrent low-grade or follicular non-Hodgkin's lymphoma. Annal Oncol 1998; 9: 995-1001.
18. Onrust SV, Lamb HM, Balfour JA. Rituximab. Drugs 1999; 58:79-88.
19. Wood AM. Rituximab: an innovative therapy for non-Hodgkin's lymphoma. Am J Health Syst Pharm 2001; 58: 215-29.
20. Zaja F, Russo D, Fuga G, Perella G, Baccarani M. Rituximab for myasthenia gravis developing after bone marrow transplant. Neurology 2000; 55:1062-3.

Peer Review Outcomes

What is already known on this topic

The use of rituximab as salvage treatment in patients with refractory autoimmune cytopenias has been reported in a limited number of cases, with promising results.

What this study adds

This study extend our information on the optimal use of this new therapy for difficult diseases in which most immunosuppressive treatments had failed to produce satisfactory outcome.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Guido Finazzi, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Finazzi and the Editors. Manuscript received November 5, 2001; accepted December 3, 2001.

Potential implications for clinical practice

Rituximab should be considered for patients with severe immune hemolytic anemia and chronic thrombocytopenia refractory to conventional treatments. Cold agglutinin disease seems more responsive than warm-antibody hemoytic anemias to the effect of the drug.

Guido Finazzi, Associate Editor