Rituximab for the treatment of autoimmune cytopenias

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B- or T-lymphocyte-mediated autoimmune disorders may lead to clinical disease characterized by low blood cell counts. The term autoimmune cytopenias has been collectively applied to this heterogeneous group of disorders. After the introduction of the humanized, chimeric monoclonal anti-CD20 antibody, rituximab, as a powerful therapeutic agent in B-cell non-Hodgkin's lymphoma,[1-3] numerous attempts have been made to utilize B-lymphocyte depletion induced by rituximab for the treatment of non-hematologic as well as hematologic autoimmune diseases.[4-6] In this issue of Haematologica, two papers address the use of rituximab as therapy for autoimmune cytopenias.[7,8]

Problems of methodology

Although a rapidly growing body of literature exists on this topic, clinical decision-making often remains difficult. The rationale for using the anti-CD20 antibody is not uniform, since the autoantibodies are produced by reactive, polyclonal B-lymphocytes in some autoimmune cytopenias[9,10] and by monoclonal cells in others.[11,12] Differences in abnormalities of T-cell subsets and antigen presentation may also contribute to the heterogeneity.[4,13]

Most autoimmune cytopenias are rare diseases, making it difficult to perform randomized trials. Even non-randomized prospective studies are relatively few, and retrospective series of consecutive patients are often small. Conclusions, therefore, have sometimes been based on pooled case reports or very small retrospective series. For instance, since 1998, numerous case reports have been published on therapy with rituximab in primary chronic cold agglutinin disease (CAD).[14-16] A small prospective trial was reported in 2001;[17] two larger phase 2 trials were published in 2004 and 2006,[18,19] and the results from a large, retrospective series appeared in 2006.[20]

By 2003, 23 cases had been published altogether and responses had been observed in 21; the two non-responders were reported in the only prospective study.[5,17,21] The two larger prospective trials and the large retrospective series, however, showed response rates between 45 and 60%.[18-20] In many case reports, remissions were classified as complete responses (CR),[14,15,21] whereas the systematic studies showed that CR to rituximab as single agent therapy are uncommon.[18-20] The obvious explanation for these major discrepancies is that response rates estimated from pooled case reports are highly likely to be influenced by publication bias and heterogeneous or poorly defined response criteria.

For most autoimmune cytopenias, it will be unrealistic to require more than two or three well-performed phase 2 trials in order to consider a given therapy modality as evidence-based. If safety and efficacy of two or more treatment modalities have been documented in a given condition, it will often be impossible to put forward evidence-based guidelines on which therapy should be preferred. Response rates derived from pooled case reports should not be accepted as a basis for clinical practice.

Autoimmune hemolytic anemia (AIHA)

Warm-antibody AIHA is idiopathic in about 50% of patients, whereas an underlying or associated disorder can be identified in the remaining 50%.[22] Even in warm-AIHA complicating clonal lymphoproliferative diseases such as chronic lymphocytic leukemia (CLL), the hemolysis is caused by high-affinity IgG antibodies produced by reactive, polyclonal B cells that have undergone somatic hypermutation.[9,23,24] Warm-antibody AIHA is frequently associated with other autoimmune diseases, suggesting a failure of regulatory mechanisms of the immune system.[24-26] An immune derangement is assumed to be present even in CLL.[27,28] Reactive, polyclonal B-lymphocytes should, therefore, be considered the primary target cells for anti-CD20 therapy in warm-type AIHA, although killing of clonal B cells may also play a role when an underlying lymphoproliferative disease is present.[28]

Case reports have indicated favorable responses to rituximab in refractory AIHA.[29-31] In a retrospective series of five patients with a variety of underlying clonal lymphoproliferative disorders, improvement was observed in all patients and responses were classified as complete in three.[32] In another retrospective study, Narat and co-authors reported on response to rituximab monotherapy in seven of 11 AIHA patients.[33] Gupta and co-workers observed good responses to combination therapy with rituximab, dexamethasone and cyclophosphamide in a retrospective study of eight patients with refractory AIHA complicating CLL.[34]

D'Arena and colleagues analyzed retrospectively the outcome after rituximab as single agent therapy in 14 CLL patients with AIHA and observed increased hemoglobin levels and transfusion independence in ten. Conversion to a negative direct antiglobulin test was achieved in five patients.[28] The same group performed a retrospective study of 11 steroid-refractory adults with idiopathic AIHA.[35] After rituximab monotherapy, all patients achieved transfusion independence and significantly increased hemoglobin levels with a mean increment of 3.3 g/dL, and remissions were classified as CR in eight patients. Favorable response rates in corticosteroid-refractory pediatric patients were observed in two retrospective[36,37] and one prospective series; in the latter, Zecca and co-authors reported responses in 13 of 15 children.[38] High response rates to rituximab monotherapy in childhood- and adult-type AIHA have also been reported from mixed retrospective series of autoimmune cytopenias.[39-41]

Primary chronic CAD accounts for 13-15% of the cases of AIHA,[22,42,43] making it an uncommon condition with a prevalence of 16 cases per million inhabitants and an incidence rate of 1 case per million per year according to a population-based study from Norway.[20] Comprehensive review articles have been published recently.[44-46]

In addition to complement-mediated immune hemolysis, most patients suffer from cold-induced circulatory symptoms, and exacerbations associated with conditions of acute phase reaction occur in more than 70% of the patients.[20,47-49] Transfusion dependence is a frequent feature.[20] Counseling on cold avoidance has been considered the mainstay of therapy, but a relatively large study showed that in more than 70% of cases, the patient and/or physician had not perceived this measure as sufficient.[20] The results of treatment with corticosteroids, azathioprine, alkylating agents, interferon-α monotherapy and cladribine monotherapy have been poor.[20,50-52] CAD is not associated with other autoimmune diseases.[20,48] The existence of clonal B cells that produce monoclonal cold agglutinins, usually IgMκ, has been postulated for decades based on electrophoretic findings[11] and such agglutinins have been verified more directly during the last decade by flow cytometric and immunohistochemical studies.[12,20]

Lymphoplasmacytic lymphoma in the bone marrow is a frequent finding even in patients otherwise classified as having primary CAD.[12,18,20] Although a role of polyclonal, reactive B-lymphocytes has been demonstrated in Waldenström's macroglobulinemia[53] and has not been excluded in primary CAD, the clonal B-lymphocytes should probably be considered the primary target cells for novel therapies.[45]

The efficiency of rituximab as single agent therapy in primary CAD has been outlined in the methodolo-

gy paragraph. The response criteria used by our group are listed in Table 1 and can be considered sufficiently strict to exclude temporary alleviation of hemolysis during higher ambient temperatures, remissions associated with recovery from infections, and clinically insignificant fluctuations of blood parameters.[17,18,52] Taken together, the Norwegian and Danish studies have shown partial responses (PR) in somewhat more than 50% of the patients.[18-20] CR, however, have been observed in only two patients (5%) among a total of 40 patients treated with rituximab monotherapy.[20] Even many patients who had received previous rituximab therapy have been shown to profit.[18] Owing to the low prevalence and incidence rate of CAD, phase 3 trials will probably never be undertaken.

Despite the favorable results of rituximab as single agent therapy in CAD, the failure rate of 40-50% and the relatively short median response duration of 11 months constitute substantial remaining problems. Small cell clones can produce biologically highly active cold agglutinins that may have to be almost completely eradicated in order to achieve clinical improvement.[18,45] Such considerations have made us and others hypothesize that rituximab-based combination therapies might show higher efficacy as compared to single agent therapy. Remission following cyclophosphamide and rituximab combination therapy has been reported in a single case,[54] but systematic studies have not been done. We are currently conducting a prospective, single-arm study of rituximab and fludarabine combination therapy.[55] The preliminary results are promising, but superiority over rituximab monotherapy remains to be proven.[56]

Autoimmune thrombocytopenia (ITP)

ITP is the most common autoimmune cytopenia.[57] Although the pathogenetic role of polyclonal antiplatelet IgG-antibodies and removal of antibody-labeled platelets by the spleen has been well documented, autoantibodies are not detectable in up to 50% of the patients.[58,59] Abnormalities of T-cell subsets have also been demonstrated.[13,60] The American as well as the British guidelines for therapy have recently been critically discussed.[61,62] As pointed out by Godeau and co-authors, both sets of guidelines are based mainly on expert opinion rather than on outcomes derived from clinical trials and should not be used as the sole basis for decisions.[62] Most authors agree that adult patients do not require treatment unless they either have a platelet count below 30×10^9/L, have bleeding symptoms, or are undergoing procedures likely to cause bleeding.[61-63] Whereas ITP tends run a chronic course in adults, childhood ITP usually resolves spontaneously after weeks or months; however, chronic disease of the adult type is encountered in 10-20% of pediatric ITP patients.[64,65]

Table 1. Response criteria in chronic cold agglutinin disease.

Response level	*Criteria*
Complete response	Absence of anemia No signs of hemolysis Disappearance of clinical symptoms of CAD Undetectable monoclonal serum protein No signs of clonal lymphoproliferation as assessed by bone marrow histology, immunohistochemistry and flow cytometry
Partial response	A stable increase in hemoglobin levels by at least 2.0 g/dL or to the normal range A reduction of serum IgM concentrations by at least 50% of the initial level or to the normal range Improvement of clinical symptoms Transfusion independence
No response	Failure to achieve complete or partial response

In order to qualify for any given response level, all criteria have to be fulfilled. The criteria have been used in most prospective studies of therapy for CAD.[17,18,52,56]

A beneficial effect of rituximab therapy in adult and childhood ITP has been shown in retrospective series[33,66,67] and prospective, single-arm trials.[68-73] The largest prospective study described in a definitive publication was done by Cooper and colleagues.[74] They assessed the safety and efficacy of rituximab therapy in 57 adults with ITP, all of whom had failed to respond to two or more previous treatment modalities. Thirty-one patients (54 %) responded; 18 had a CR and 13 a PR. Fifteen of 18 patients who achieved a CR maintained their response for more than 1 year, while response duration was generally short in patients achieving a PR. A preliminary report of an equally sized, prospective single-arm trial of rituximab in adult ITP has been provided by Godeau and co-authors, who observed very good responses in 24 of 60 patients (40%) and therapy failure in 34 (57%).[75] At least 18 patients (30%) maintained a good response for more than 1 year. Several case reports have described response to re-treatment with rituximab in ITP patients who relapsed after previous rituximab therapy.[76]

Pre-defined criteria for CR and PR were used in most reports; however, there was some variation in the platelet count thresholds.[7,70,74,77] The most commonly used criteria are listed in Table 2. In a recent, comprehensive literature analysis, Arnold and colleagues calculated that according to pooled results of studies enrolling at least five patients, responses were achieved in 63% of the patients, with a 95% confidence interval (CI) of 53-73%; the responses were complete in 46% (CI, 30-58%); and partial in 24% (CI, 15-38%).[77]

While these studies clearly document the therapeutic efficacy of rituximab in chronic, refractory ITP in

both adults and children, we still do not know which ITP patients should receive rituximab therapy. In contrast to CAD, in which the relatively efficient treatment with rituximab can be compared to several ineffective conventional therapies even in the absence of comparative phase 3 studies, further trials are definitively needed in order to determine the place of rituximab in the treatment of ITP. Corticosteroids (or in some cases high-dose immunoglobulin) still hold their position as first-line therapy,[62,78] and splenectomy remains a relatively efficient and sufficiently safe second-line treatment.[62,79] Numerous options exist or may be proposed for third-line treatment, including rituximab and the new thrombopoietin receptor agonists.[80] As compared to splenectomy, however, rituximab therapy is a non-surgical and reversible measure with a favorable safety profile and reasonably high response rates.

The question has, therefore, been raised as to whether infusions of rituximab should be preferred to splenectomy as a second-line treatment, possibly reducing the need for splenectomy. Clearly, randomized studies are warranted in order to try to answer this question, and it is hoped that the relatively high incidence rate of ITP will permit the completion of well-designed trials. Such studies are currently being performed.[81,82]

Evans' syndrome

Evans' syndrome is a rare condition defined by the combination of AIHA and ITP in the absence of any underlying disease.[83,84] Typically, the disease runs a chronic course in both adults and children and is characterized by frequent exacerbations and remissions. Although direct antiglobulin test is almost invariably positive, most often showing IgG on the erythrocyte surface, T-cell abnormalities have also been a consistent finding.[84,85] Management remains difficult. Corticosteroids are still the mainstay of first-line treatment, but relapses during tapering are common. As for second-line therapy, there are many options but few systematic studies to support their use.[7,84] Rituximab therapy has previously been described in at least 19 cases.

Ten patients were reported as single case observations;[86-89] four were described within a mixed retrospective series of autoimmune cytopenias;[39] and a prospective series of childhood AIHA included five patients with Evans' syndrome.[38] Thus, all previously published reports should be regarded as case observations that do not allow calculation of response rates. Nevertheless, remission after infusions of rituximab was achieved in many of these cases. The brief report in this issue of Haematologica by Bader-Meunier and co-authors, describing a French retrospective multicenter series of rituximab therapy in 17 children with refractory Evans' syndrome is, therefore, of considerable interest.[7] The retrospective design may have posed some methodological problems. Furthermore, it was probably not easy to define adequate response criteria, since response definitions for AIHA as well as ITP had to be taken into consideration and might be a matter of discussion. Anyway, this is a large study of therapy in such a rare disease. CR or PR of at least one cytopenia was achieved in 13 patients (76%), with no obvious difference in responses between the AIHA and ITP components. Among ten patients who presented with AIHA and ITP simultaneously, remission of both cytopenias was observed in six. As the authors conclude, rituximab therapy should be considered at an early stage in children with steroid-refractory Evans' syndrome. Further prospective trials should be performed at a multi-national level, if possible.

Table 2. Response criteria in autoimmune thrombocytopenia.

Response level	*Platelet count ($x10^9$/L)*	*Additional criteria*
Complete response	> 150	
Partial response	50-150	In order to qualify for partial response, at least a 2-fold increase should be achieved
No response	< 50	

The platelet count criteria have been used in most recent series and in a comprehensive, pooled literature analysis.[7,70,74,77] The additional criterion for PR was proposed in order to exclude non-responders presenting with insignificant increases in platelet count.[7]

Other autoimmune cytopenias

All original publications on rituximab therapy for autoimmune neutropenia (AIN) and pure red cell aplasia (PRCA) are case reports[37,90-92] or descriptions of small numbers of patients within mixed series of autoimmune cytopenias.[40,41,93] Success has been reported in single cases of PRCA complicating CLL,[91,92] AIN in immunodeficient children,[40] and AIN combined with ITP.[90] Results of rituximab therapy have been disappointing, however, in several other cases of AIN and PRCA.[93] A more important role of T-cell abnormalities as compared to humoral autoimmunity has been proposed as an explanation,[93,94] although such statements are uncertain in these heterogeneous and rare conditions.

Tolerability and dosage

The favorable safety profile of rituximab therapy has been clearly documented in lymphoma patients, despite the well-known, rather frequent infusion-related adverse effects and the less frequent delayed toxicity.[2,95,96] The amount of B-lymphocytes killed after administration of rituximab is less in autoimmune dis-

orders than in lymphoma. In our experience, rituximab toxicity is a small problem in patients with CAD.[17,18] Conflicting results have been published regarding the frequency and severity of adverse events in polyclonal autoimmune cytopenias,[7,41,69,73,74,77] possibly reflecting more frequent side effects in children.[7,97] Generally, however, literature and experience indicate that adverse effects are tolerable, reversible and probably less frequent than in lymphoma patients. Rapid (90-minute) infusion of rituximab has been shown to be a safe option in corticosteroid-pretreated lymphoma patients receiving their second or later infusion,[98] and trials exploring the safety of this regimen may be justified in patients with autoimmune cytopenias as well.

Since response duration may be relatively short and re-treatment with rituximab may be effective even several times,[18,20] severe B-cell immunodeficiency might be a possible concern. In follicular lymphoma, however, prolonged rituximab therapy has not resulted in a significantly increased incidence of adverse events.[99] Furthermore, although peripheral B-lymphocytes are virtually absent after weekly administration of four doses, naïve and memory B cells can usually be detected again after 4-6 months.[100] In my experience with CAD patients, even repeated treatment with rituximab has been well tolerated.[18,45]

Dose-finding trials of rituximab therapy have hardly been performed in autoimmune cytopenias, and the dosage as well as treatment schedule used in follicular lymphoma has been adopted almost uniformly.[2,3,18,74] In this issue of the journal, the question of dosage is addressed by Provan and colleagues.[8] They analyzed retrospectively the data of 11 consecutive patients with autoimmune cytopenias who received a fixed dose of 100 mg rituximab weekly for 4 weeks. All patients had failed to respond to conventional therapies. The authors observed sustained CR in four of seven patients with ITP and in one patient with pancytopenia, and concluded that low dose rituximab offers a further treatment option in autoimmune cytopenias. Larger, confirmatory studies are required. If the results can be confirmed, rituximab therapy for autoimmune cytopenias will become substantially cheaper and may be associated with even less toxicity than today.

Conclusions

The literature on rituximab therapy for autoimmune cytopenias is growing rapidly. Nevertheless, the low prevalence and incidence rate of most of these diseases makes it difficult to perform large, prospective studies that can underpin evidence-based guidelines. The most convincing documentation has been provided in CAD and ITP, and a reasonable amount of evidence has been provided in warm-antibody AIHA. In CAD, rituximab may currently be recommended as first-line treatment in patients who require medical therapy, but further trials are warranted in order to improve on response rate and duration. In ITP and warm-antibody AIHA, further studies are needed in order to put forward recommendations on which patients should receive rituximab therapy. The recent data on rituximab for the treatment of Evans' syndrome are promising. Preliminary results seem less encouraging in AIN and PRCA. In these two conditions, more established therapy modalities should be preferred in clinical practice until more data have been provided.

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, Bodkin DJ, White CA, Liles TM, Royston I, et al. IDEC-C2B8: results of a phase I multiple-dose trial in patients with relapsed non-Hodgkin's lymphoma. J Clin Oncol 1997;15:3266-74.
3. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, et al. 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;16:2825-33.
4. Silverman GJ. Anti-CD20 therapy and autoimmune disease: therapeutic opportunities and evolving insights. Front Biosci 2007;12:2194-206.
5. Finazzi G. Rituximab in autoimmune cytopenias: for which patients? Haematologica 2002;87:113-4.
6. Robak T. Monoclonal antibodies in the treatment of autoimmune cytopenias. Eur J Haematol 2004;72:79-88.
7. Bader-Meunier B, Aladjidi N, Bellmann F, Monpoux F, Nelken B, Robert A, et al. Rituximab therapy for childhood-Evans syndrome. Haematologica 2007;92:In press.
8. Provan D, Butler T, Evangelista ML, Amadori S, Newland AC, Stasi R. Activity and safety profile of low-dose rituximab for the treatment of autoimmune cytopenias in adults. Haematologica 2007;92:In press.
9. Efremov DG, Ivanovski M, Burrone OR. The pathologic significance of the immunoglobulins expressed by chronic lymphocytic leukemia B-cells in the development of autoimmune hemolytic anemia. Leuk Lymphoma 1998;28:285-93.
10. He R, Reid DM, Jones CE, Shulman NR. Spectrum of Ig classes, specificities, and titers of serum antiglycoproteins in chronic idiopathic thrombocytopenic purpura. Blood 1994;83:1024-32.
11. Harboe M, Deverill J. Immunochemical properties of cold haemagglutinins. Scand J Haematol 1964;61:223-37.
12. Berentsen S, Bo K, Shammas FV, Myking AO, Ulvestad E. Chronic cold agglutinin disease of the "idiopathic" type is a premalignant or low-grade malignant lymphoproliferative disease. APMIS 1997;105:354-62.
13. Stasi R, Del Poeta G, Stipa E, Evangelista ML, Trawinska MM, Cooper N, et al. Response to B-cell depleting therapy with rituximab reverts the abnormalities of T cell subsets in patients with idiopathic thrombocytopenic purpura. Blood 2007;110:2924-30. Epub 2007 Jun 4.
14. Lee EJ, Kueck B. Rituxan in the treatment of cold agglutinin disease. Blood 1998;92:3490-1.
15. Cohen Y, Polliack A, Zelig O, Goldfarb A. Monotherapy with rituximab induces rapid remission of recurrent cold agglutinin-mediated hemolytic anemia in a patient with indolent lympho-plasmacytic lymphoma. Leuk Lymphoma 2001;42:1405-8.
16. Layios N, Van Den NE, Jost E, Deneys V, Scheiff JM, Ferrant A. Remission of severe cold agglutinin disease after rituximab therapy. Leukemia 2001;15:187-8.
17. Berentsen S, Tjonnfjord GE, Brudevold R, Gjertsen BT, Langholm R, Lokkevik E, et al. Favourable response to therapy with the anti-CD20 monoclonal antibody ritux-

imab in primary chronic cold agglutinin disease. Br J Haematol 2001;115:79-83.
18. Berentsen S, Ulvestad E, Gjertsen BT, Hjorth-Hansen H, Langholm R, Knutsen H, et al. Rituximab for primary chronic cold agglutinin disease: a prospective study of 37 courses of therapy in 27 patients. Blood 2004;103:2925-8.
19. Schollkopf C, Kjeldsen L, Bjerrum OW, Mourits-Andersen HT, Nielsen JL, Christensen BE, et al. Rituximab in chronic cold agglutinin disease: a prospective study of 20 patients. Leuk Lymphoma 2006;47:253-60.
20. Berentsen S, Ulvestad E, Langholm R, Beiske K, Hjorth-Hansen H, Ghanima W, et al. Primary chronic cold agglutinin disease: a population based clinical study of 86 patients. Haematologica 2006;91:460-6.
21. Camou F, Viallard JF, Pellegrin JL. [Rituximab in cold agglutinin disease]. Rev Med Interne 2003;24:501-4.
22. Dacie J. The auto-immune haemolytic anaemias: introduction. In: Dacie J, ed. The Haemolytic Anaemias, Vol. 3. London: Churchill Livingstone; 1992: pp. 1-5.
23. Fanning LJ, Connor AM, Wu GE. Development of the immunoglobulin repertoire. Clin Immunol Immunopathol 1996;79:1-14.
24. Fagiolo E. Immunological tolerance loss vs. erythrocyte self antigens and cytokine network disregulation in autoimmune hemolytic anaemia. Autoimmun Rev 2004;3:53-9.
25. Steinman L. Escape from "horror autotoxicus": pathogenesis and treatment of autoimmune disease. Cell 1995; 80:7-10.
26. Tan EM. Autoantibodies: what do they recognize? Verh Dtsch Ges Pathol 1996;80:1-11.
27. Diehl LF, Ketchum LH. Autoimmune disease and chronic lymphocytic leukemia: autoimmune hemolytic anemia, pure red cell aplasia, and autoimmune thrombocytopenia. Semin Oncol 1998;25:80-97.
28. D'Arena G, Laurenti L, Capalbo S, D'Arco AM, De Filippi R, Marcacci G, et al. Rituximab therapy for chronic lymphocytic leukemia-associated autoimmune hemolytic anemia. Am J Hematol 2006;81:598-602.
29. Lee E, Zamkoff KW, Gentile TC, Zimrin A. Rituxan in the treatment of auto-immune hemolytic anemia (AIHA) [42nd annual meeting of the American Society of Hematology, San Francisco, USA. Abstract 2560]. Blood 2000;96:596a-7a[abstract].
30. 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.
31. Perrotta S, Locatelli F, La Manna A, Cennamo L, De Stefano P, Nobili B. Anti-CD20 monoclonal antibody (Rituximab) for life-threatening autoimmune haemolytic anaemia in a patient with systemic lupus erythematosus. Br J Haematol 2002;116:465-7.
32. Trape G, Fianchi L, Lai M, Laurenti L, Piscitelli R, Leone G, et al. Rituximab chimeric anti-CD20 monoclonal antibody treatment for refractory hemolytic anemia in patients with lymphoproliferative disorders. Haematologica 2003;88:223-5.
33. Narat S, Gandla J, Hoffbrand AV, Hughes RG, Mehta AB. Rituximab in the treatment of refractory autoimmune cytopenias in adults. Haematologica 2005;90:1273-4.
34. Gupta N, Kavuru S, Patel D, Janson D, Driscoll N, Ahmed S, et al. Rituximab-based chemotherapy for steroid-refractory autoimmune hemolytic anemia of chronic lymphocytic leukemia. Leukemia 2002;16:2092-5.
35. D'Arena G, Califano C, Annunziata M, Tartarone A, Capalbo S, Villani O, et al. Rituximab for warm-type idiopathic autoimmune hemolytic anemia: a retrospective study of 11 adult patients. Eur J Haematol 2007;79:53-8.
36. Quartier P, Brethon B, Philippet P, Landman-Parker J, Le Deist F, Fischer A. Treatment of childhood autoimmune haemolytic anaemia with rituximab. Lancet 2001;358: 1511-3.
37. 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.
38. Zecca M, Nobili B, Ramenghi U, Perrotta S, Amendola G, Rosito P, et al. Rituximab for the treatment of refractory autoimmune hemolytic anemia in children. Blood 2003;101:3857-61.
39. Shanafelt TD, Madueme HL, Wolf RC, Tefferi A. Rituximab for immune cytopenia in adults: idiopathic thrombocytopenic purpura, autoimmune hemolytic anemia, and Evans syndrome. Mayo Clin Proc 2003;78: 1340-6.
40. Kim JJ, Thrasher AJ, Jones AM, Davies EG, Cale CM. Rituximab for the treatment of autoimmune cytopenias in children with immune deficiency. Br J Haematol 2007; 138:94-6.
41. Rao A, Kelly M, Musselman M, Ramadas J, Wilson D, Grossman W, et al. Safety, efficacy, and immune reconstitution after rituximab therapy in pediatric patients with chronic or refractory hematologic autoimmune cytopenias. Pediatr Blood Cancer 2007.
42. Genty I, Michel M, Hermine O, Schaeffer A, Godeau B, Rochant H. [Characteristics of autoimmune hemolytic anemia in adults: retrospective analysis of 83 cases]. Rev Med Interne 2002;23:901-9.
43. Sokol RJ, Hewitt S, Stamps BK. Autoimmune haemolysis: an 18-year study of 865 cases referred to a regional transfusion centre. Br Med J (Clin Res Ed) 1981;282:2023-7.
44. Berentsen S, Beiske K, Tjonnfjord G. Primary chronic cold agglutinin disease: an update on pathogenesis, clinical features and therapy. Hematology 2007;12:361-70.
45. Berentsen S, Ulvestad E, Tjonnfjord G. B-lymphocytes as targets for therapy in chronic cold agglutinin disease. Cardiovasc Hematol Disord Drug Targets 2007;7:219-27.
46. Gertz MA. Management of cold haemolytic syndrome. Br J Haematol 2007;138:422-9.
47. Ulvestad E. Paradoxical haemolysis in a patient with cold agglutinin disease. Eur J Haematol 1998;60:93-100.
48. Ulvestad E, Berentsen S, Bo K, Shammas FV. Clinical immunology of chronic cold agglutinin disease. Eur J Haematol 1999;63:259-66.
49. Ulvestad E, Berentsen S, Mollnes TE. Acute phase haemolysis in chronic cold agglutinin disease. Scand J Immunol 2001;54:239-42.
50. Dacie J. Treatment and prognosis of cold-antibody AIHA. In: Dacie J, ed. The Haemolytic Anaemias, Vol. 3. London: Churchill Livingstone; 1992: pp. 502-8.
51. Hillen HF, Bakker SJ. Failure of interferon-alpha-2b therapy in chronic cold agglutinin disease. Eur J Haematol 1994;53:242-3.
52. Berentsen S, Tjonnfjord GE, Shammas FV, Bergheim J, Hammerstrom J, Langholm R, et al. No response to cladribine in five patients with chronic cold agglutinin disease. Eur J Haematol 2000;65:88-90.
53. Kriangkum J, Taylor BJ, Treon SP, Mant MJ, Belch AR, Pilarski LM. Clonotypic IgM V/D/J sequence analysis in Waldenstrom macroglobulinemia suggests an unusual B-cell origin and an expansion of polyclonal B cells in peripheral blood. Blood 2004;104:2134-42.
54. Vassou A, Alymara V, Chaidos A, Bourantas KL. Beneficial effect of rituximab in combination with oral cyclophosphamide in primary chronic cold agglutinin disease. Int J Hematol 2005;81:421-3.
55. Berentsen S, Tjonnfjord GE, Ulvestad E. Therapy for chronic cold agglutinin disease: a prospective, non-randomized international multicenter study on the safety and efficacy of rituximab in combination with fludarabine. http://www.clinicaltrials.gov/ct/show/NCT00373594?order=1. 2006. Accessed on 14 Sept 2007.
56. Berentsen S, Tjonnfjord GE. Rituximab and fludarabine combination therapy for chronic cold agglutinin disease Haematologica. 2006;91(Suppl 1):11. [11th congress of the European Hematology Association, Amsterdam, abstract 0027].
57. Frederiksen H, Schmidt K. The incidence of idiopathic thrombocytopenic purpura in adults increases with age. Blood 1999;94:909-13.
58. Warner MN, Moore JC, Warkentin TE, Santos AV, Kelton JG. A prospective study of protein-specific assays used to investigate idiopathic thrombocytopenic purpura. Br J Haematol 1999;104:442-7.

59. Cines DB, Blanchette VS. Immune thrombocytopenic purpura. N Engl J Med 2002;346:995-1008.
60. Bennett CM, de Jong JL, Neufeld EJ. Targeted ITP strategies: do they elucidate the biology of ITP and related disorders? Pediatr Blood Cancer 2006;47:706-9.
61. British Committee for Standards in Haematology General Haematology Task Force. Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy. Br J Haematol 2003;120:574-96.
62. Godeau B, Provan D, Bussel J. Immune thrombocytopenic purpura in adults. Curr Opin Hematol 2007; 14:535-56.
63. George JN. Management of patients with refractory immune thrombocytopenic purpura. J Thromb Haemost 2006;4:1664-72.
64. Franchini M, Zaffanello M, Veneri D, Lippi G. Rituximab for the treatment of childhood chronic idiopathic thrombocytopenic purpura and hemophilia with inhibitors. Pediatr Blood Cancer 2007;49:6-10.
65. Tamminga RY, Bruin MC. Rituximab treatment for symptomatic chronic ITP. Pediatr Blood Cancer 2006; 47:714-6.
66. Penalver FJ, Jimenez-Yuste V, Almagro M, Alvarez-Larran A, Rodriguez L, Casado M, et al. Rituximab in the management of chronic immune thrombocytopenic purpura: an effective and safe therapeutic alternative in refractory patients. Ann Hematol 2006;85:400-6.
67. Parodi E, Nobili B, Perrotta S, Rosaria Matarese SM, Russo G, Licciardello M, et al. Rituximab (anti-CD20 monoclonal antibody) in children with chronic refractory symptomatic immune thrombocytopenic purpura: efficacy and safety of treatment. Int J Hematol 2006; 84:48-53.
68. 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.
69. Giagounidis AA, Anhuf J, Schneider P, Germing U, Sohngen D, Quabeck K, et al. Treatment of relapsed idiopathic thrombocytopenic purpura with the anti-CD20 monoclonal antibody rituximab: a pilot study. Eur J Haematol 2002;69:95-100.
70. Zaja F, Vianelli N, Sperotto A, de Vita S, Iacona I, Zaccaria A, et al. B-cell compartment as the selective target for the treatment of immune thrombocytopenias. Haematologica 2003;88:538-46.
71. Wang J, Wiley JM, Luddy R, Greenberg J, Feuerstein MA, Bussel JB. Chronic immune thrombocytopenic purpura in children: assessment of rituximab treatment. J Pediatr 2005;146:217-21.
72. Taube T, Schmid H, Reinhard H, von Stackelberg A, Overberg US. Effect of a single dose of rituximab in chronic immune thrombocytopenic purpura in childhood. Haematologica 2005;90:281-3.
73. Bennett CM, Rogers ZR, Kinnamon DD, Bussel JB, Mahoney DH, Abshire TC, et al. Prospective phase 1/2 study of rituximab in childhood and adolescent chronic immune thrombocytopenic purpura. Blood 2006;107: 2639-42.
74. Cooper N, Stasi R, Cunningham-Rundles S, Feuerstein MA, Leonard JP, Amadori S, et al. The efficacy and safety of B-cell depletion with anti-CD20 monoclonal antibody in adults with chronic immune thrombocytopenic purpura. Br J Haematol 2004;125:232-9.
75. Godeau B, Fain O, Porcher R, Lefere F, Fenaux P, Cheze S, et al. Rituximab is an alternative to splenectomy in adults with chronic immune thrombocytopenic purpura: results of a multicenter prospective phase 2 study. [48th annual meeting of the American Society of Hematology, Orlando, FL, USA. 478]. Blood 2006;108:45[Abstract].
76. Perrotta AL. Re-treatment of chronic idiopathic thrombocytopenic purpura with rituximab: literature review. Clin Appl Thromb Hemost 2006;12:97-100.
77. Arnold DM, Dentali F, Crowther MA, Meyer RM, Cook RJ, Sigouin C, et al. Systematic review: efficacy and safety of rituximab for adults with idiopathic thrombocytopenic purpura. Ann Intern Med 2007;146:25-33.
78. Stevens W, Koene H, Zwaginga JJ, Vreugdenhil G. Chronic idiopathic thrombocytopenic purpura: present strategy, guidelines and new insights. Neth J Med 2006; 64:356-63.
79. Kojouri K, Vesely SK, Terrell DR, George JN. Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. Blood 2004;104:2623-34.
80. Bussel JB, Kuter DJ, George JN, McMillan R, Aledort LM, Conklin GT, et al. AMG 531, a thrombopoiesis-stimulating protein, for chronic ITP. N Engl J Med 2006;355:1672-81.
81. Ghanima W, Holme PA. Rituximab as second line treatment for ITP. http://www.clinicaltrials.gov/ct/show/NCT00344149?order=1. 2007. Accessed on 13 Sept 2007.
82. Arnold DM, Crowther MA. Pilot study of rituximab for the treatment of acute immune thrombocytopenic purpura (ITP). http://www.clinicaltrials.gov/ct/show/NCT00372892?order=3. 2007. Accessed on 13 Sept 2007.
83. Evans RS, Takahashi K, Duane RT, Payne R, Liu C. Primary thrombocytopenic purpura and acquired hemolytic anemia; evidence for a common etiology. AMA Arch Intern Med 1951;87:48-65.
84. Norton A, Roberts I. Management of Evans syndrome. Br J Haematol 2006;132:125-37.
85. Wang W, Herrod H, Pui CH, Presbury G, Wilimas J. Immunoregulatory abnormalities in Evans syndrome. Am J Hematol 1983;15:381-90.
86. Seipelt G, Bohme A, Koschmieder S, Hoelzer D. Effective treatment with rituximab in a patient with refractory prolymphocytoid transformed B-chronic lymphocytic leu kemia and Evans syndrome. Ann Hematol 2001;80:170-3.
87. Galor A, O'Brien T. Rituximab treatment for relapsed autoimmune hemolytic anemia in Evans syndrome. Int J Hematol 2003;78:335-6.
88. Jubinsky PT, Rashid N. Successful treatment of a patient with mixed warm and cold antibody mediated Evans syndrome and glucose intolerance. Pediatr Blood Cancer 2005;45:347-50.
89. Park CY, Chung CH. A patient with mixed type Evans syndrome: efficacy of rituximab treatment. J Korean Med Sci 2006;21:1115-6.
90. Faurschou M, Hasselbalch HC, Nielsen OJ. Sustained remission of platelet counts following monoclonal anti-CD20 antibody therapy in two cases of idiopathic autoimmune thrombocytopenia and neutropenia. Eur J Haematol 2001;66:408-11.
91. Batlle M, Ribera JM, Oriol A, Plensa E, Milla F, Feliu E. Successful response to rituximab in a patient with pure red cell aplasia complicating chronic lymphocytic leukaemia. Br J Haematol 2002;118:1192-3.
92. Ghazal H. Successful treatment of pure red cell aplasia with rituximab in patients with chronic lymphocytic leukemia. Blood 2002;99:1092-4.
93. Dungarwalla M, Marsh JC, Tooze JA, Lucas G, Ouwehand W, Pettengell R, et al. Lack of clinical efficacy of rituximab in the treatment of autoimmune neutropenia and pure red cell aplasia: implications for their pathophysiology. Ann Hematol 2007;86:191-7.
94. Willis F, Marsh JC, Bevan DH, Killick SB, Lucas G, Griffiths R, et al. The effect of treatment with Campath-1H in patients with autoimmune cytopenias. Br J Haematol 2001;114:891-8.
95. Davis TA, Grillo-Lopez AJ, White CA, McLaughlin P, Czuczman MS, Link BK, et al. Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of re-treatment. J Clin Oncol 2000;18:3135-43.
96. Grillo-Lopez AJ. Monoclonal antibody therapy for B-cell lymphoma. Int J Hematol 2002;76:385-93.
97. Larrar S, Guitton C, Willems M, Bader-Meunier B. Severe hematological side effects following rituximab therapy in children. Haematologica 2006;91:ECR36.
98. Sehn LH, Donaldson J, Filewich A, Fitzgerald C, Gill KK,

Runzer N, et al. Rapid infusion rituximab in combination with corticosteroid-containing chemotherapy or as maintenance therapy is well tolerated and can safely be delivered in the community setting. Blood 2007;109: 4171-3.
99. Ghielmini M, Schmitz SF, Cogliatti SB, Pichert G, Hummerjohann J, Waltzer U, et al. Prolonged treatment with rituximab in patients with follicular lymphoma significantly increases event-free survival and response duration compared with the standard weekly x 4 schedule. Blood 2004;103:4416-23.
100. Wirths S, Ruprecht C, Bertoni F, Kanz L, Lanzavecchia A. Hierachy of human B cell regeneration after rituximab therapy [48th annual meeting of the American Society of Hematology, Orlando, Abstract 936]. Blood 2006;108: 279a[Abstract].