**Tolerance and short-term efficacy of rituximab in 43 patients with systemic autoimmune diseases**

Gottenberg JE[1], Guillevin L[2], Lambotte O[3], Combe B[4], Allanore Y[5], Cantagrel A[6], Larroche C[7], Soubrier M[8], Bouillet L[9], Dougados M[10], Fain O[11], Farge D[12], Kyndt X[13], Lortholary O[14], Masson C[15], Moura B[16], Remy P[17], Thomas T[18], Wendling D[19], Anaya JM[20], Sibilia J[21], Mariette X[1*], for the Club Rhumatismes et Inflammation (CRI).

[1]Service de Rhumatologie, Hôpital de Bicêtre, Assistance Publique des Hôpitaux de Paris (AP-HP), Le Kremlin-Bicêtre, France
[2]Service de Médecine Interne, Hôpital Cochin, AP-HP, Paris, France
[3]Service de Médecine Interne, Hôpital de Bicêtre, AP-HP, Le Kremlin-Bicêtre, France
[4]Service d' Immuno- Rhumatologie, Hôpital Lapeyronie, Montpellier, France
[5]Service de Rhumatologie A, Hôpital Cochin, AP-HP, Paris, France
[6]Service de Rhumatologie, Centre Hospitalier Universitaire (CHU) Rangueil, Toulouse, France
[7]Service de Médecine Interne, Hôpital Avicenne, AP-HP, Bobigny, France
[8]Service de Rhumatologie, CHU Gabriel Montpied, Clermont-Ferrand, France
[9]Service de Médecine Interne, CHU de Grenoble, Grenoble, France
[10]Service de Rhumatologie B, Hôpital Cochin, AP-HP, Paris, France
[11]Service de Médecine Interne, Hôpital Jean Verdier, AP-HP, Bondy, France
[12]Service de Médecine Interne, Hôpital Saint-Louis, AP-HP, Paris, France
[13]Service de Nephrologie et de Médecine Interne, CHU de Valenciennes, Valenciennes, France
[14]Service des Maladies Infectieuses et Tropicales, Hôpital Necker-Enfants Malades, AP-HP, Paris, France
[15]Service de Rhumatologie, CHU d'Angers, Angers, France
[16]Service de Rhumatologie, Hôpital Ambroise Paré, AP-HP, Boulogne, France
[17]Service de Nephrologie, Hôpital Henri Mondor, AP-HP, Créteil, Francer
[18]Service de Rhumatologie, CHU de Saint-Etienne, Saint-Etienne, France
[19]Service de Rhumatologie, CHU Jean Minjoz, Besancon, France
[20]Unidad de Reumatología CIB, Universidad Pontificia Bolivariana, Medellín, Colombia
[21]Service de Rhumatologie, Hôpital Hautepierre, Strasbourg, France

[*]Correspondence and reprint requests to Pr Xavier MARIETTE, Service de Rhumatologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin Bicêtre
Tel: +33 1 45 21 37 58, Fax: +33 1 45 21 37 57
E-mail: xavier.mariette@bct.ap-hop-paris.fr

**Running Title**: Treatment with rituximab in patients with autoimmune diseases.

**Key words:** rituximab, rheumatoid arthritis, systemic lupus erythematosus, primary Sjögren's syndrome, vasculitis.

## ABSTRACT

**Objective**. To assess the tolerance and efficacy of rituximab in patients with various autoimmune diseases seen in daily rheumatological practice.
**Patients and Methods**. Eight hundred and sixty-six rheumatology and internal medicine practitioners were contacted by E-mail to obtain the files of patients treated with rituximab for systemic autoimmune diseases. Patients with lymphoma were analysed if the evolution of the autoimmune disease could be evaluated.
**Results**. A total of 43 of 49 cases could be analysed in this retrospective study, including 14 patients with rheumatoid arthritis (RA), 13 with systemic lupus erythematosus (SLE), 6 with primary Sjögren's syndrome (pSS), 5 with systemic vasculitis and 5 with other autoimmune diseases. Rituximab was prescribed for lymphoma in 2 patients with RA and 2 with pSS. In the 39 other cases, rituximab was given because of the refractory character of the autoimmune disease. The mean follow-up period was 8.3 months (2–26 months). Eleven adverse events were observed in 10 patients and treatment had to be discontinued in 6 patients. The efficacy of rituximab was observed in 30 patients (70%): 11 RA, 9 SLE, 5 pSS, 2 vasculitis, 2 antisynthetase syndromes and 1 sarcoidosis. The mean decrease in daily corticosteroid intake was 9.5 mg/day (0-50) in responders. Seven patients experienced relapse after 8.1 months (5-15), on average. Three patients died because of refractory autoimmune disease.
**Conclusion**. Despite the absence of marketing authorization, rituximab is used to treat various refractory autoimmune diseases in daily rheumatological practice. The present study shows good tolerance, short-term clinical efficacy and marked corticosteroid reduction with rituximab therapy in patients with RA, SLE, pSS, vasculitis or polymyositis.

After decades during which the T cell was considered the cornerstone of autoimmunity, interest has recently grown in the pivotal role of B cells in autoantibody secretion, autoantigen presentation (1), pro-inflammatory cytokine production (2) and regulation of dendritic cell function (3). Thus, rituximab, a chimeric monoclonal antibody specific for human CD20, which targets B lymphocytes, could be a potential new biological treatment of autoimmune diseases. To date, rheumatoid arthritis (RA) is the only disease in which efficacy of rituximab was demonstrated in a controlled trial (4). Because rituximab received marketing authorization for lymphoma and despite the absence of marketing authorization in autoimmune diseases, some clinicians have already started to use it to treat refractory autoimmune diseases.

The Club Rhumatismes et Inflammation (CRI), a section of the Société Française de Rhumatologie, has 866 members from approximately 100 departments of rheumatology and internal medicine registered on its Web site, which allows for collecting reliable data on practice in France regarding autoimmune diseases. The present retrospective study was initiated by the CRI to evaluate the safety and efficacy of rituximab in patients with diverse autoimmune diseases.

## PATIENTS AND METHODS

### Patient selection

Eight hundred and sixty-six rheumatology and internal medicine practitioners registered on the Web site of CRI were contacted 4 times by E-mail to obtain the files of patients treated with rituximab for the following systemic autoimmune diseases: RA, systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS) and vasculitis. The charts were reviewed by 2 of the authors (XM and JEG). The diagnoses were re-assessed by use of international consensus criteria (American College of Rheumatology for RA, SLE and vasculitis and the European-American consensus group criteria for pSS). Patients with lymphoma were analysed if the evolution of the autoimmune disease could be evaluated. It was ensured that each patient whithout lymphoma had been informed that the treatment was experimental and had not received marketing authorization in autoimmune diseases. After these detailed explanations, each patient gave informed consent for treatment with rituximab.

### Treatment

The number of rituximab infusions, as well as the dosage and rhythm of infusion, was recorded in each patient. Concomitant medications, including immunosuppressant agents and prednisone, were noted.

### Assessment

Tolerance and adverse events were recorded for each patient. Disease activity score 28 (DAS28) and SLE disease activity index (SLEDAI) values were recorded in patients with RA and SLE, respectively. In patients with RA or SLE, efficacy was defined as a decrease of 50% (partial remission [PR]) or more of the initial DAS28 and SLEDAI values, respectively. Patients with RA and a DAS value below 2.6 were considered to be in complete remission (CR) as well as patients with SLE and a SLEDAI value between 0 and 2. In patients with other autoimmune diseases, efficacy was defined as a decrease of 50% (PR) or more of the initial disease activity according to the clinician in charge of the patient. Patients with other autoimmune diseases, in whom all symptoms and clinical features disappeared, were considered to be in CR.

## RESULTS

### Patient characteristics

Twenty-four departments of rheumatology and internal medicine had patients treated with rituximab for autoimmune diseases. Forty-nine observations were collected. Six were excluded because of diagnosis uncertainty (n = 3) or inability to evaluate the course of the autoimmune disease in patients with lymphoma (n = 3). Thus, 43 observations could be analysed. The clinical characteristics of the 43 patients are reported in Tables 1-5. The study included 14 patients with RA, 13 with SLE, 6 with pSS, 5 with systemic vasculitis and 5 with other inflammatory arthritides. (antisynthetase syndrome [n = 2], systemic sclerosis [n = 1], Still's disease [n = 1], sarcoidosis [n = 1]). Rituximab was prescribed for lymphoma associated with autoimmune disease in 2 patients with RA and 2 with pSS. In the 39 other cases, rituximab was given because of the refractory character of the autoimmune disease. Nine patients with RA had previously been treated with anti-TNF, 7 SLE patients with cyclophosphamide, 2 type II mixed cryoglobulinemia patients with interferon and ribavirin and 3 patients with vasculitis had not responded to cyclophosphamide and infliximab.

### Characteristics of rituximab administration and concomitant medications

Thirty-five patients received weekly infusions of 375 mg/m$^2$ of rituximab for 4 weeks, 5 patients were given 2 infusions of 1000 mg, and a different dosage regimen was followed in 3 patients (patients 3, 5 and 38, Tables 1 and 4). Rituximab was given in combination with methotrexate (MTX) in 6 patients, with other immunosuppressants in 15 patients, with pulses of methylprednisolone in 7 patients and prescribed alone in 15 patients (Tables 1-5). The mean follow-up period was 8.3 months (2–26 months).

### Tolerance and safety

Eleven adverse events were observed in 10 patients. Infusion-related reactions, such as transient hypo- or hypertension, were observed in 3 patients (patients 10, 33 and 35). These infusion-related reactions occurred during the first infusion in 2 patients (patients 10 and 33), who had experienced prior infusion-related reactions with infliximab, and during the seventh infusion (third infusion after clinical relapse) in the other (patient 35). In one patient with chronic hepatitis C (patient 34) and renal function impairment (creatinine clearance of 35 mL/mn), 2 repeated episodes of encephalopathy developed 2 days after the second and third infusion, concomitantly with hyperammonemia (2.18 and 4.17 mg/L, respectively, N<0.8 mg/L). This patient had histologic features of chronic hepatitis, without cirrhosis. Two patients with SLE (patients 22 and 24) and one with pSS (patient 29) had serum sickness-like reactions (urticaria, arthralgias within 2 days after the first and third infusions (patient 22) and after each infusion (patients 24 and 29)). In 1 patient with SLE-related end-stage renal disease requiring hemodialysis (patient 15), severe sepsis-like syndrome along with neutropenia (blood neutrophil count 0.6 x $10^9$/L) developed 10 days after a 500-mg infusion of rituximab. One patient with SLE had neutropenia (blood neutrophil count 0.7 x $10^9$/L) 15 days after the first infusion of rituximab (patient 26). Mycophenolate mofetil, which had been given concomitantly (1.5 g/day), was discontinued, and the blood neutrophil count returned to within the normal range. Two patients with SLE, including 1 with nephrotic syndrome (patient

21), developed deep vein thrombosis, with pulmonary embolism in 1 (patient 22), 5 and 4 months, respectively, after treatment with rituximab. Both patients had IgG anticardiolipin antibodies.

Treatment was discontinued in 6 of these 11 patients. Late-onset neutropenia and marked hypogammaglobulinemia were not observed, but serum gammaglobulin levels were monitored in only 21 patients. Liver enzyme levels were initially 1.5 and 2 as high as normal in 2 patients with hepatitis C viral (HCV) replication (patients 34 and 35) and did not significantly change after rituximab therapy. Liver enzyme levels remained within the normal range in the other patients.

**Short-term efficacy of anti-CD20 therapy**

Efficacy of rituximab was observed in 30 patients (70%), including 11 RA, 9 SLE, 5 pSS, 2 vasculitis, 2 antisynthetase syndromes and 1 sarcoidosis. Efficacy was observed in 17 of these patients not concomitantly with DMARDs/immunosuppressants, 5 with MTX and 8 with other DMARDS/immunosuppressants. Mean time to response was 5.4 weeks (1-16). The mean decrease of daily corticosteroid dose was 9.5 mg/day (0-50) in patients who responded to rituximab. Oral corticosteroids could be discontinued in 4 patients (patients 5,12, 31 and 33). Seven patients, including 5 patients treated with rituximab alone (patients 2, 16, 35, 39 and 41) and 2 patients treated with MTX and cyclosporin (patients 3 and 23, respectively), experienced relapse after 8.1 months (5-15), on average. Four patients were retreated with rituximab, but the current follow-up is not sufficient to evaluate the clinical evolution of these patients.
Three patients died because of refractory autoimmune disease (rheumatoid vasculitis, refractory autoimmune haemolytic anaemia in a SLE patient and refractory cerebral vasculitis in another SLE patient).

*Patients with RA* (Table 1). Eleven of 14 patients with RA responded to rituximab, including 4 patients in complete remission (DAS28 < 2.6) and 7 patients in partial remission (decrease of 50% or more of the initial DAS28). The mean DAS28 decreased from 6.7±1.3 (5.3-8.7) to 3.4±1.4 (1.8-6.7) ($P < 0.0002$ using paired t-test). In 11 responders, the mean daily dosage of prednisone decreased from 9.3 mg (0-20) to 5.9 mg (0-10) ($P < 0.01$) after a mean follow-up duration of 8.6 months (2-24). Rheumatoid factor values, evaluated in 8 RF-positive patients, decreased in 6 patients (Table 1).

*Patients with SLE* (Table 2). Nine of 13 patients with SLE responded to rituximab, including 7 patients in complete remission (SLEDAI value between 0 and 2) and 2 patients in partial remission (decrease of 50% or more of the initial SLEDAI). Two patients died because of the refractory character of the autoimmune disease. Two of the 4 SLE patients with active nephritis and 7 of the 9 patients without nephritis responded to rituximab. The mean SLEDAI value decreased from 11±7 (3-28) to 5±6 (0-20) in the 11 surviving patients ($P < 0.0002$ using paired t-test). For the 9 responders, the mean daily dosage of prednisone decreased from 27 mg (5-60) to 8 mg (2-13) ($P < 0.01$), after a mean follow-up duration of 9.8 months (4-26). The variation in the level of anti-double-stranded DNA antibody was different in individual patients (Table 2).

*Patients with pSS* (Table 3). The efficacy of rituximab and partial remission were observed in 5 of 6 patients, with regression of parotid swelling, articular

involvement, subjective dryness and fatigue in 2 patients (patients 32 and 33), improvement of subjective dryness in 1 (patient 28), and major improvement of cryoglobulinemia-related vasculitis in 2 (patients 30 and 31), with concomitant disappearance of cryoglobulinemia. In 1 patient with salivary lymphoma of the MALT subset (patient 29), therapy with rituximab was not successful. Regarding glandular symptoms, self-reported dryness was improved in only 3 patients (patients 28, 32 and 33) and was not changed in the 3 others, whereas objective ocular and mouth dryness, assessed in only patients 28 and 30, was not improved. RF levels decreased in the 3 responders in whom it was assessed but did not decrease in the patient, who was non responder to rituximab (Table 3). In all patients with anti-SSA/SSB antibodies (4 patients with anti-SSA antibody, 2 with anti-SSB), these autoantibodies remained detectable after rituximab.

*Patients with vasculitis* (Table 4). Efficacy of rituximab was observed in 2 of 5 patients, in whom partial remission was obtained. One patient with rheumatoid vasculitis died 6 weeks after rituximab because of acute respiratory distress syndrome, which was not due to alveolar hemorrhage (patient 36). One patient with cirrhosis, treated with rituximab for HCV-related type II mixed cryoglobulinemia, healed from skin ulcers and had no detectable cryoglobulinemia 5 months after the last infusion (patient 35). Peripheral nerve and renal involvement remained stable despite rituximab. The mixed cryoglobulinemia reappeared 7 months after the last infusion of rituximab, and the patient experienced a clinical relapse (purpura flares) 5 months later. One patient with Wegener's granulomatosis (patient 38), who had been non responder to MTX and mycophenolate mofetil (MMF), experienced a marked response to rituximab: ear, nose and throat involvement was stabilized, and a concomitant pyoderma gangrenosum was markedly improved. Anti-proteinase 3 antibody, determined by ELISA (initial serum level of 90 IU), disappeared within 6 months of treatment.

*Patients with other inflammatory arthritides* (Table 5). Polymyositis was greatly improved in 2 patients treated with rituximab for antisynthetase syndrome (patients 39 [partial remission] and 40 [complete remission]). The creatine kinase (CK) level of patient 39 decreased from 252 to 119 IU/L (N <150). This patient, treated with rituximab alone, also experienced good improvement of pemphigus-associated lesions; mucosal lesions disappeared, and skin lesions were markedly reduced. The CK level of patient 40 decreased from 1364 to 93 IU/L (N<160) and serum aldolase decreased from 25 to 8 (N<7.5). Muscle strength normalised in this patient, allowing for a marked decrease of daily prednisone. The patient was able to go back to work 4 months after rituximab therapy. Anti-Jo1 antibody remained detectable in both patients.

Rituximab alone had a dramatic clinical effect on cervical lymphadenopathy as well as a clear corticosteroid sparing effect in 1 patient with lymph node sarcoidosis (patient 41), who achieved CR after treatment.

No efficacy of rituximab was observed in 1 patient treated with rituximab and cyclophosphamide for severe Still's disease and polysynovitis (patient 42). Rituximab and fludarabin had no effect on the clinical course of systemic sclerosis in 1 patient who had previously experienced relapse after high-dose cyclophosphamide and autologous stem-cell transplantation (patient 43).

**Monitoring of B cell depletion**

B-cell depletion (defined as a peripheral CD19+ B lymphocyte count < 5 cells/µl), which was assessed in only 12 patients, was observed in all but 1 patient, in whom rituximab therapy was unsuccessful (patient 29). Two of the 11 remaining patients, whose B-cell pool was depleted, did not respond to rituximab (patients 13 and 17).

**Monitoring of HCV load**

Three patients had antibodies against hepatitis C, including 2 with cryoglobulinemia-related vasculitis (patients 34 and 35) and 1 with SLE (patient 22). HCV load was not detectable in patient 22 before treatment and remained undetectable after rituximab. In the two patients with cryoglobulinemia, HCV viral load increased shortly after rituximab and then returned to base line (Table 4).

## DISCUSSION

This retrospective study demonstrates that despite the absence of marketing authorization, rituximab is used in various refractory autoimmune diseases in daily rheumatological practice. The use of rituximab for off-label autoimmune diseases might even be more frequent, since this retrospective study depended on the recall of clinicians to accure cases. Short-term efficacy could be observed in 70% of patients. Additionally, rituximab therapy allowed for a reduction of oral corticosteroids by 9.5 mg/day, on average, in responders. Limitations to this retrospective study include the heterogeneity of the diseases studied and the rather short follow-up duration, which prevents from drawing definite conclusions regarding the long-term efficacy of rituximab. This led us to concentrate mainly on the tolerance of rituximab in these 43 patients with autoimmune diseases.

Tolerance of rituximab was good and in accordance with data from the literature. Interestingly, 2 patients (patients 10 and 35) with infusion-related reactions experienced the same side effects with infliximab, another chimeric monoclonal antibody. In these patients, a cross-allergy to the mice component could be hypothesized. The 2 patients, who experienced the most severe side effects (encephalopathy and sepsis-like syndrome), had renal function impairment. Serum sickness-like reactions, which occurred repeatedly in 3 patients have been described in patients treated with rituximab (5-7). The 3 deaths appeared to be a consequence of the refractory character of the autoimmune disease and not to intolerance of rituximab.

The main experience of rituximab in autoimmune diseases comes from a controlled trial in RA (4). In that study, 43% (rituximab and MTX) and 41% (rituximab and cyclophosphamide) of patients with RA achieved 50% improvement according to the ACR criteria, compared with 13% of patients given MTX alone. Our results compare well with that of the controlled trial, showing comparable efficacy and tolerance of rituximab in patients not under trial conditions, including 9 patients in failure of anti-TNF therapy.

The first published open study (8) of SLE included 6 patients with severe SLE treated with rituximab, high-dose steroids and cyclophosphamide, which led to clinical improvement in 5 patients. The same group reported the results of 6 patients with nephritis: four of them experienced a marked improvement of lupus activity and of serologic and renal features (9). Recently, another group reported the efficacy of rituximab added to current therapy in 18 patients (10). Clinical activity of lupus markedly decreased in 10 patients in whom B cells were depleted. No overall change in serum levels of anti-dsDNA antibodies was observed. A few case reports exist of treatment with rituximab for autoimmune hemolytic anemia (11), thrombocytopenia (12), and neurological involvement with secondary anti-phospholipid syndrome (13) in patients with SLE.

In the present study, two of the 4 SLE patients with active nephritis and 7 of the 9 without nephritis responded to rituximab. These results suggest the potential use of rituximab in severe SLE, since 2 of 4 patients with lupus nephritis and 1 patient with central nervous system involvement were greatly improved. Additionally, rituximab had a marked corticosteroid sparing effect in milder but corticodependent forms of lupus with cutaneous or articular involvement, since it allowed for decreasing the daily prednisone dosage in responders by more than 3 times on

average. No conclusion regarding the effect of rituximab on anti-dsDNA level can be drawn given the limited number of patients assessed.

In pSS, the first published observation described self-reported subjective improvement of oral and ocular dryness in pSS following therapy with rituximab for parotid marginal zone lymphoma (14). Recently, the clinical evolution of 4 patients treated with rituximab for pSS-associated lymphoma was reported (15). Type II mixed cryoglobulinemia (MC), observed in 3 patients, disappeared. No information was given concerning the evolution of sicca syndrome. In the present study, extraglandular symptoms, notably swelling of the parotid gland, arthralgias, and cryoglobulinemia-related vasculitis, were sensitive to rituximab in all but 1 patient. Decrease in level of RF and/or cryoglobulinemia accompanied clinical improvement of extraglandular signs in responders. However, no conclusion can be drawn regarding the effect of rituximab on dryness.

For patients with vasculitis and HCV-related type II MC, the largest experience comes from 2 Italian studies, involving 15 and 20 patients each (16, 17). A significant increase in level of HCV RNA was observed in responders in 1 of the series, as in 2 of our patients (including 1 responder). Thus, the association of anti-viral therapy to rituximab could be considered in patients with replicating hepatitis C. The present study involved 4 patients with cryoglobulinemia (2 HCV-related, 2 pSS-related). Rituximab had a great effect on purpura and skin ulcers in 3 patients and peripheral nerve involvement in 2. Cryoglobulinemia disappeared in the 3 responders. Concerning ANCA-related vasculitis, only 1 case of Wegener's granulomatosis (WG), with good response to rituximab in combination with cyclophosphamide and high-dose steroids, was reported (18). The present study involved 2 patients with WG, in whom treatment with CPH had been unsuccessful. Only one patient responded well to rituximab, with a concomitant marked improvement of pyoderma gangrenosum and disappearance of anti-proteinase 3 antibody.

Clinicians involved in the study also used rituximab for other inflammatory arthritides such as antisynthetase syndrome, sarcoidosis, systemic sclerosis and Still's disease, for which no case reports have been reported. Rituximab could be a promising therapeutic strategy in antisynthetase syndrome, since it normalized muscular strength and muscle enzyme levels and reduced corticosteroid dosage in our 2 patients. Likewise, rituximab has shown good efficacy in 5 patients with refractory dermatomyositis (19). Efficacy of rituximab in sarcoidosis, observed in 1 patient, also requires further study.

Interestingly, almost half of the responders received rituximab concomitantly with immunosuppressants. Among the 7 patients who experienced clinical relapse of the disease, 5 patients had taken rituximab alone. Thus, in autoimmune diseases, combination therapy might improve the efficacy of rituximab, as demonstrated in RA (4), and might delay the occurrence of relapses.

B-cell count decrease paralleled clinical efficacy in 9 patients and was stable in 1 patient in whom therapy with rituximab was unsuccessful. In patients who experience relapse, clinical manifestation of disease is often preceded by the reappearance of B cells and/or autoantibodies (20), which could be observed in 3 patients (reappearance of B cells in 1 patient with SLE, of anti-dsDNA antibodies in another patient with SLE and of cryoglobulinemia in 1 patient with HCV-related vasculitis).

This study showed good tolerance, short-term clinical efficacy and marked reduction in corticosteroid use with rituximab treatment in patients with various refractory autoimmune diseases. Controlled trials should be carried out to

demonstrate the therapeutic effect of rituximab in SLE, pSS, vasculitis and polymyositis.

## ACKNOWLEDGEMENTS

We thank Olivier Meyer (Hôpital Bichat, AP-HP, Paris, France) and Christophe Richez (CHU de Bordeaux, Bordeaux, France) for their collaboration.
We are indebted to Stephanie Poulain (CHU de Valenciennes, Valenciennes, France) and Arielle Rosenberg (Hôpital Cochin, AP-HP, Paris, France) for HCV viral load assessment.

REFERENCES

1. Mamula MJ, Fatenejad S, Craft J. B cells process and present lupus autoantigens that initiate autoimmune T cell responses. J Immunol 1994;152:1453-61.
2. Harris DP, Haynes L, Sayles PC, Duso DK, Eaton SM, Lepak NM, et al. Reciprocal regulation of polarized cytokine production by effector B and T cells. Nat Immunol 2000;1:475-82.
3. Moulin V, Andris F, Thielemans K, Maliszewski C, Urbain J, Moser M. B lymphocytes regulate dendritic cell (DC) function in vivo: increased interleukin 12 production by DCs from B cell-deficient mice results in T helper cell type 1 deviation. J Exp Med 2000;192:475-82.
4. Edwards JC, Szczepanski L, Szechinski J, Filipowicz-Sosnowska A, Emery P, Close DR, et al. Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. N Engl J Med 2004;350:2572-81.
5. D'Arcy CA, Mannik M. Serum sickness secondary to treatment with the murine-human chimeric antibody IDEC-C2B8 (rituximab). Arthritis Rheum 2001;44:1717-8.
6. Herishanu Y. Rituximab-induced serum sickness. Am J Hematol 2002;70:329.
7. Hellerstedt B, Ahmed A. Delayed-type hypersensitivity reaction or serum sickness after rituximab treatment. Ann Oncol 2003;14:1792.
8. Leandro MJ, Edwards JC, Cambridge G, Ehrenstein MR, Isenberg DA. An open study of B lymphocyte depletion in systemic lupus erythematosus. Arthritis Rheum 2002;46:2673-7.
9. Leandro MJ EM, Edwards JCW, Manson J, Cambride G, Isenberg DA. Treatment of refractory lupus nephritis with B lymphocyte depletion. Arthritis Rheum 2003;48:S378:924.
10. Looney RJ AJ, Campbell D, Felgar RE, Young F, Arend LJ. B cell depletion as a novel treatment for systemic lupus erythematosus. A phase I/II dose-escalation trial of rituximab. Arthritis Rheum 2004;50:2580-2589.
11. 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.
12. ten Cate R, Smiers FJ, Bredius RG, Lankester AC, van Suijlekom-Smit LW, Huizinga TW, et al. Anti-CD20 monoclonal antibody (rituximab) for refractory autoimmune thrombocytopenia in a girl with systemic lupus erythematosus. Rheumatology 2004;43:244.
13. Weide R, Heymanns J, Pandorf A, Koppler H. Successful long-term treatment of systemic lupus erythematosus with rituximab maintenance therapy. Lupus 2003;12:779-82.
14. Somer BG, Tsai DE, Downs L, Weinstein B, Schuster SJ. Improvement in Sjogren's syndrome following therapy with rituximab for marginal zone lymphoma. Arthritis Rheum 2003;49:394-8.
15. Voulgarelis M, Giannouli S, Anagnostou D, Tzioufas AG. Combined therapy with rituximab plus cyclophosphamide/doxorubicin/vincristine/prednisone (CHOP) for Sjogren's syndrome-associated B-cell aggressive non-Hodgkin's lymphomas. Rheumatology 2004;43:1050-1053.
16. Sansonno D, De Re V, Lauletta G, Tucci FA, Boiocchi M, Dammacco F. Monoclonal antibody treatment of mixed cryoglobulinemia resistant to interferon alpha with an anti-CD20. Blood 2003;101:3818-26.

17. Zaja F, De Vita S, Russo D, Michelutti A, Fanin R, Ferraccioli G, et al. Rituximab for the treatment of type II mixed cryoglobulinemia. Arthritis Rheum 2002;46:2252-4.
18. Specks U, Fervenza FC, McDonald TJ, Hogan MC. Response of Wegener's granulomatosis to anti-CD20 chimeric monoclonal antibody therapy. Arthritis Rheum 2001;44:2836-40.
19. Levine TD. A pilot study for rituximab therapy. Arthritis Rheum 2002;46:S488:1299.
20. Cambridge G, Leandro MJ, Edwards JC, Ehrenstein MR, Salden M, Bodman-Smith M, et al. Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. Arthritis Rheum 2003;48:2146-54.

**Table 1**. Demographic and clinical data of 14 patients treated with rituximab for RA.

Duration: disease duration; MP: methylprednisolone; IS: immunosuppressants; pred: prednisone; RF: rheumatoid factor (determined by nephelometry, except

| Patients | Sex/ age/ duration of disease (years) | No. of prior DMARDS/ prior anti-TNF (Y/N) | Non-articular involvement (Y/N) | No. infusions X dose (mg)/ No. MP pulses (mg) / concomitant IS | Adverse event (Y/N) | Efficacy RA/non-articular involvement (Y/N) | Time to response (weeks)/ follow-up (months) | DAS28 initial/ last[2] | Pred initial/ last[3] (mg/d) | RF initial/ last (IU/mL) (N<20) | Relapse (Y/N)/ time to relapse (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F/48/4 | 8/Y | N | 4X375ms/HQ | N | Y | 4/6 | 5.9/2.9 | 15/10 | pos/NA | N |
| 2 | M/83/3 | 3/Y | Y/lymph | 4X375ms/4 MP (100) | N | Y/Y | 6/5.5 | 7.6/2.4 | 12/9 | NA | Y/5.5 |
| 3 | F/42/15 | 11/Y | N | 27X375ms/MTX,CPH | N | Y | 16/15 | 8.3/3.6 | 10/10 | 270/150 | Y/5 |
| 4 | F/47/2 | 4/Y | N | 2X1000/2 MP (100) /MTX+HQ | N | Y | 4/3 | 8.7/4.7 | 0/0 | 240/122 | N |
| 5 | F/68/18 | 3/N | Y/lymph | 8X1000/8 CHOP | N | Y/Y | 4/24 | 5.3/2.7 | 3/0 | 266/20 | N |
| 6 | M/57/7 | 3/Y | N | 2X1000/CPH | N | Y | 4/5 | 6/3.2 | 20/10 | 938/441 | N |
| 7 | F/41/8 | 5/Y | N | 2X1000/MTX | N | N | -/4 | 6/5.2 | 10/10 | 95/NA | - |
| 8 | M/55/12 | 6/Y | N | 2X1000 | N | Y | 4/8 | 7.9/3.6 | 17.5/10 | pos/neg | N |
| 9 | F/48/7 | 5/N | N | 2X1000/MTX | N | Y | 8/12 | 4/1.8 | 10/2.5 | neg/neg | |
| 10 | M/49/3 | 7/Y | N | 1X375ms/1 MP(100) | infusion-related | N | -/3 | NA | 10/10 | pos/pos | - |
| 11 | F/68/34 | 5/Y | N | 4X375ms/4 MP(40) | N | Y | 4/3 | 6.6/3.8 | 4/4 | NA | N |
| 12 | F/53/3 | 2/N | N | 4X375ms/4 MP (40) | N | Y | 6/6 | 6.5/2.2 | 4/0 | 20/20 | N |
| 13[1] | M/67/2 | 1/N | Y/Felty | 4X375ms | N | N/N | -/6 | 6.6/6.7 | 15/15 | neg/neg | - |
| 14[1] | M/53/4 | 2/N | Y/Felty | 4X375ms/MTX | N | Y/N | 4/12 | 7.5/2.1 | 15/10 | 60/14 | N |

RF stated as «pos/neg», determined by latex); pos : positive; neg : negative; F: female; M: male; lymph: lymphoma; ms: square meter; HQ: hydroxychloroquine; MTX: methotrexate; CPH: cyclophosphamide; NA: not available; - : not relevant.[1]: evolution of neutropenia of patients of 13 and 14 was described in a submitted case report.

[2]*: P* < 0.0002 between DAS28 value pre and post treatment using paired t-test.[3]: *P* < 0.01 between prednisone dose pre and post treatment.

**Table 2**. Demographic and clinical data of 13 patients treated with rituximab for SLE.

| Patients | Sex/ age/ duration (years) | No. prior IS/ prior CPH (Y/N) | Clinical involvement | No. infusions X dose (mg)/ No. of MP pulses (mg)/ concomitant IS | Adverse event (Y/N) | Efficacy (Y/N) | Time to response (weeks)/ follow-up (months) | SLEDAI initial/ last[1] | Pred initial/ last (mg/d)[2] | Anti-dsDNA IgG initial/last ELISA(IU/mL) (N <20 ) | C3 and/or C4 normalisation (Y/N) | Relapse (Y/N)/ time to relapse (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | F/30/3 | 1/N | pleuro-pericarditis | 1X375ms | neutropenia | N | -/6 | 8/6 | 25/25 | 40/neg[*] | N | - |
| 16 | M/20/7 | 3/Y | nephritis/myocarditis/ Evans | 4X375ms | N | Y | 2/15 | 18/6 | 60/10 | 144/10[*] | Y | Y/15 |
| 17 | F/55/8 | 2/N | auto-immune haemolytic anemia, lymphadenopathy | 4X375ms/ 2 MP(80)/ iv Ig | N | death | -/2 | 8/NA | 60/15 | neg/neg | N | - |
| 18 | M/21/3 | 2/Y | CNS (psychosis) | 4X375ms | N | Y | 12/26 | 9/2 | 50/2 | 25/neg[*] | Y | N |
| 19 | F/26/10 | 3/N | skin, articular | 4X375ms | N | Y | 4/6 | 6/0 | 30/6 | 99/0 | Y | N |
| 20 | F/41/5 | 2/N | articular | 4X375ms/ AZA | N | Y | 1/7 | 6/2 | 5/2 | neg/neg | NA | N |
| 21 | F/30/13 | 3/Y | articular, vasculitis, nephritis | 4X375ms/HQ | deep vein thrombosis | N | -/5 | 28/20 | 50/15 | 100-500 | N | - |
| 22 | F/28/19 | 2/Y | skin, articular | 4X375ms | serum-sickness, pulmonary embolism | Y | 4/4 | 6/2 | 14/13 | 1280-320 | N | N |
| 23 | M/33/9 | 5/Y | auto-immune thrombocytopenia, nephritis | 4X375ms/ CYA | N | Y | 8/9 | 8/2 | 30/10 | NA/31 | Y | Y/9 |
| 24 | F/28/18 | 3/N | auto-immune thrombocytopenia, APS | 3X375ms/ AZA | serum-sickness | Y | 4/10 | 3/2 | 30/10 | 300/286 | N | N |
| 25 | F/28/2 | 3/Y | nephritis, thrombotic microangiopathy | 4X375ms/ plasmaph/CPH | N | death | -/2 | 24/NA | 60/60 | 113/NA | N | - |
| 26 | F/22/3 | 3/Y | articular | 4X375ms/ 2 MP(80)/ MMF | neutropenia | Y | 6/7 | 9/0 | 12.5/10 | 199/neg | Y | N |
| 27 | F/30/20 | 2/N | articular, vasculitis | 4X375ms | N | Y | 4/4 | 18/14[1] | 9/8 | 50/75 | N | N |

Duration: disease duration; CPH: cyclophosphamide; MP: methylprednisolone; IS: immunosuppressants; ms: square meter; CNS: central nervous system; APS: anti-phospholipid syndrome; iv Ig: intravenous immunoglobulins; MMF: mycophenolate mofetil; HQ: hydroxychloroquine; AZA: azathioprine; CYA: cyclosporin; plasmaph: plasmapheresis; pos: positive; neg: negative; NA: not available; -: not relevant; [*]: Farr's test (N<7); [1] : a marked improvement of digital angeitis was observed and proteinuria resolved. [1]: $P < 0.0002$ between SLEDAI value pre and post treatment using paired t-test. [2]: $P < 0.01$ between prednisone dose pre and post treatment.

**Table 3**. Demographic, clinical and biological data of 6 patients treated with rituximab for primary Sjögren's syndrome.

| Patients | Sex/ age/ duration (years) | Prior IS | Clinical involvement at beginning of rituximab treatment | No. infusions X dose (mg)/ no. of MP pulses (mg)/ concomitant IS | Adverse event (Y/N) | Efficacy on extraglandular involvement (Y/N) | Initial/final VAS | Efficacy on objective dryness (Y/N) | Time to response (weeks)/ follow-up (months) | Pred initial/ last | RF(IU/L) intial/last (N<20) | Relapse (Y/N)/ time to relapse (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 28 | F/58/15 | HQ | digestive lymphoma(MALT) | 4X375ms/ 4MP(500)/HQ | N | Y | dryness VAS: 80/50 | stable Schirmer | 4/6 | 9/6 | 44/0 | N |
| 29 | F/43/4 | No | salivary lymphoma (MALT) | 4X375ms | serum-sickness | N | NA | NA | -/11 | 0/0 | 499/423 | - |
| 30 | F/71/5 | HQ/CPH/AZA | vasculitis | 4X375ms | N | Y | NA | stable Schirmer, salivary flow = 0 | 8/8 | 15/7.5 | RF: 109/57 cryo: 1%/neg | N |
| 31 | F/58/2 | CPH | vasculitis | 4X375ms | N | Y | NA | NA | 8/8 | 10/0 | RF: 170/neg cryo: pos/neg | N |
| 32 | F/74/18 | MTX/ETA/INF | parotid gland enlargement, polysynovitis | 4X375ms/ 4 MP(40) | N | Y | dryness VAS: 60/20 fatigue VAS: 70/0 | NA | 4/7 | 6/6 | RF: 130/NA | N |
| 33 | F/41/8 | CPH | parotid gland enlargement, polyarthralgias | 2X375ms/ 2 MP(40) | infusion-related | Y | dryness VAS: 20/0 fatigue VAS: 80/0 | NA | 4/7 | 4/0 | NA | N |

Duration: disease duration; IS: immunosuppressants; ms: square meter; MP: methylprednisolone; VAS: visual analogical scale; pred: prednisone; RF: rheumatoid factor (determined by nephelometry); HQ: hydroxychloroquine; CPH: cyclophosphamide; AZA: azathioprine; ETA: etanercept; Inflix: infliximab; MTX: methotrexate; cryo: cryoglobulinemia; pos: positive; neg: negative; NA: not available; -: not relevant.

**Table 4**. Demographic, clinical and biological data of 5 patients treated with rituximab for systemic vasculitis.

| | Disease | Sex/ age/ duration (years) | Prior IS | Clinical involvement at beginning of rituximab treatment | No. infusions X dose (mg)/ no. of MP pulses (mg)/ concomitant IS | Adverse event (Y/N) | Efficacy (Y/N) | Time to response (weeks)/ follow-up (months) | Pred initial/ last | Auto-Ab initial/last | HCV load (intial/ M3/M12) (logIU/mL) | Relapse (Y/N)/ time to relapse (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 34 | Type II MC | F/53/2 | IFN+Ribav | renal | 3X375ms/ 3 MP (1000) | encephalo-pathy | N | -/9 | 20/7.5 | cryo: pos/pos RF: pos/pos | 6.4/ 7.3/ 6.3 | - |
| 35 | Type II MC | F/66/18 | IFN+Ribav/ Inflix | skin, nerve, renal | 4X375ms | infusion-related | Y | 4/20 | 20/5 | cryo: (mg/L) 1104/neg RF: 289/neg | 5.7/ 6.5/ 5.1 | Y/13 |
| 36 | Rheumatoid vasculitis | F/38/11 | HQ/MTX/CPH/ plasmaph/Inflix | nerve, myocardial, intestinal | 3X375ms/ 2 MP(500)/ CPH | N | death | -/1.5 | 60/60 | NA | - | - |
| 37 | Wegener's syndrome | M/31/1 | CPH/AZA Inflix/MTX | ENT, lung | 3X375ms/ 3 MP(200) plasmaph | N | N | -/9 | 15/15 | cANCA: pos/pos | - | - |
| 38 | Wegener's syndrome | M/60/13 | CPH/iv Ig/ AZA/MMF/ Inflix | ENT; Pyoderma Gangrenosum | 15X375ms/ MTX+MMF | N | Y | 4/24 | 5/5 | anti-PR3: (ELISA) 90 IU/neg | - | N |

Duration: disease duration; MP: methylprednisolone; IS: immunosuppressants; pred: prednisone; auto-ab: autoantibody; MC: mixed cryoglobulinemia; HQ: hydroxychloroquine; CPH: cyclophosphamide; AZA: azathioprine; MTX: methotrexate; IFN: interferon; Ribav: ribavirin; Inflix: infliximab; iv Ig: intravenous immunoglobulins; MMF: mycophenolate mofetil; plasmaph: plasmapheresis; ENT: eye-nose-throat; RF: rheumatoid factor(IU/mL, determined by nephelometry, N<20); cryo: cryoglobulinemia; anti-PR3: anti-proteinase 3 antibody; pos : positive ; neg : negative ; NA: not available; -: not relevant .

**Table 5**. Demographic, clinical and biological data of 5 patients treated with rituximab for diverse autoimmune diseases.

| Patients | Disease | Sex/ age/ duration (years) | Prior IS | Clinical involvement at beginning of rituximab treatment | No. infusions X dose (mg)/ no. of MP pulses (mg)/ concomitant IS | Adverse event (Y/N) | Efficacy (Y/N) | Time to response (weeks)/ follow-up (months) | Pred initial/ last | Auto-Ab initial/last | Relapse (Y/N)/ time to relapse (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 39 | Anti-synthetase syndrome | F/55/19 | ALC/HQ/ iv Ig/ MTX | Polymyositis/ pemphigus | 4X375ms | N | Y | 4/7 | 20/18 | Anti-Jo1: pos/pos | Y/4 |
| 40 | Anti-synthetase syndrome | F/53/6 | MTX/AZA/ Iv Ig | Polymyositis | 4X375ms | N | Y | 4/5 | 20/10 | Anti-Jo1: pos/pos | N |
| 41 | Sarcoidosis | M/37/17 | No | Cervical and mesenteric lymphadenopathy | 4X375ms | N | Y | 8/11 | 20/10 | - | Y/5 |
| 42 | Still's disease | F/32/15 | MTX/Inflix HQ/ CYA | Polysynovitis | 2X375ms/ 2 MP(1000)/ CPH | N | N | -/2 | 100/100 | - | - |
| 43 | Systemic sclerosis | M/57/6 | CPH/SCT/ Fluda | Systemic sclerosis | 5X375ms/ Fluda | N | N | -/5 | neg/neg | Anti-SCL70: pos/pos | - |

Duration: disease duration; MP: methylprednisolone; IS: immunosuppressants; pred: prednisone; auto-ab: autoantibody; ALC: allochrysine; HQ: hydroxychloroquine; CPH: cyclophosphamide; AZA: azathioprine; MTX: methotrexate; iv Ig: intravenous immunoglobulins; CYA: cyclosporin; Fluda: fludarabin; SCT: autologous stem-cell transplantation; pos: positive; neg: negative; -: not relevant.