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A 4-year observation in lupus nephritis patients treated with an intensified B-lymphocyte depletion without immunosuppressive maintenance treatment—Clinical response compared to literature and immunological re-assessment

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Abstract

Background

B cells (BC) play a critical role in systemic lupus erythematosus (SLE). BC depletion therapy still remains an attractive option, despite the disappointing results of randomized controlled trials (RCTs).

Methods

Twelve patients with SLE [3 males, mean age 43.8 yrs (25–55)] with severe multiorgan involvement all including kidney (3 patients with Class IV, 4 with Class III/V and 5 with Class V, according to the International Society of Nephrology/Renal Pathology Society glomerulonephritis classification), skin lesions [10], severe polyarthralgias with arthritis [10], polyserositis [2], and lymphadenopathy [5] have been prospectively treated with an intensified B cell depletion therapy (IBCDT) protocol due to their resistance or intolerance to previous therapy (six cases) or as a front line immunosuppressive treatment in 6 women with unsatisfactory therapeutic compliance or as a specific request of a short-time immunosuppression for gestational perspectives. Protocol: Rituximab (RTX) 375 mg/sm on days 1, 8, 15, 22, and 2 more doses after 1 and 2 months, associated with 2 IV administrations of 10 mg/kg of cyclophosphamide and 3 methylprednisolone pulses (15 mg/kg) followed by oral prednisone (0.8 mg/kg/day, rapidly tapered to 5 mg/day by the end of the 3rd month after RTX). No further immunosuppressive maintenance therapy has been given.

Results

Patients had been followed-up for a mean of 44.5 (24–93) months. Significant decreases ($p < 0.05$) were found in the levels of ESR (baseline mean value: 55.0 mm; 3 months: 36; end of follow-up: 13), anti-dsDNA antibodies (baseline: 185 U; 3 months: 107; end of follow-up: 15), and proteinuria (baseline: 4.9 g/24 h; 3 months: 0.97; end of follow-up: 0.22). C4 values (baseline 11 mg/dl) significantly increased ($p < 0.05$) after 3 months (22 mg/dl) and at the end of the follow-up (20 mg/dl). Of the 12 patients, 9 (75%) have remained well after one cycle of IBCDT, with no flare (mean 51.6 months [25–93]). Three patients relapsed after 36, 41, and 72 months, respectively. Following re-treatment, they again showed complete remission over 18–48 months of observation.

Conclusions

A promising role of RTX in an intensified protocol of induction therapy can be envisaged in patients for whom avoiding immunosuppressive maintenance therapy and sparing steroids are particularly appealing. Moreover, our data confirm in one of the longest follow-up available, the opportunity to reconsider the regimens of BL depletion in the treatment of the most severe or refractory forms of SLE despite the disappointing results of RCTs.

Abbreviations

RTX, rituximab; IS, immunosuppression; GC, glucocorticoid; F/U, follow-up; PCS, prospective color study; RCS, retrospective cohort study; CR, complete remission; PR, partial remission; LN, lupus nephritis; NM, not mentioned; CYC, cyclophosphamide; RCT, randomized controlled trials; MTP, methylprednisolone; AZA, azathioprine; MMP, mycophenolate; Cys, cyclosporine; UTI, urinary tract infection

1. Introduction

B cells are thought to play an important role in the pathogenesis of systemic lupus erythematosus (SLE) [1] and [2]. B cell depletion therapy (BCDT), based on rituximab (RTX), a chimeric monoclonal antibody specific for CD20, has proved to be promising in the treatment of patients with SLE [3] and other autoimmune conditions [4], [5], [6], [7], [8], [9] and [10]. We have previously published the favorable outcome of 8 patients with severe SLE treated with an intensive short-term treatment with RTX, cyclophosphamide, and methylprednisolone pulses [11]. Our approach was able to avoid further immunosuppressive maintenance therapy [12].

BCDT is generally well tolerated, but its long-term safety profile is still debatable as most studies have follow-up data of less than 1 year.

We are now reporting on the very long-term outcome of 12 prospectively enrolled patients with renal involvement SLE (the original cohort plus an additional 7 patients) treated with the intensified BCDT (IBCDT) at our center.

Additionally, the observed results were compared to those emerging from the updated reviews of the literature on this topic focusing on study with a minimum follow-up of 24 months.

2. Methods

2.1. Patients

Twelve patients, nine women and three males, mean age 43.8 years (range 25–55 years), with severe multiorgan involvement, including kidney (3 patients with Class IV, including a case of rapidly progressive glomerulonephritis, with 60% of florid crescents), 4 with Class III/V and 5 with Class V Lupus Nephritis (LN according to the International Society of Nephrology/Renal Pathology Society Glomerulonephritis Classification), skin lesions [10 patients], severe polyarthralgias with arthritis [10], polyserositis [2], and lymphadenopathy [5], were considered eligible for RTX therapy due to their resistance or intolerance to previous therapy (6 cases) or as a front line treatment in 6 women with unreliable therapeutic compliance or gestational perspectives.

In non-naïve patients, prior immunosuppressive therapy included methylprednisolone pulses and oral steroids (all 6 previously treated patients), I.V. cyclophosphamide (2 patients), and both intravenously and orally administered cyclophosphamide for a cumulative dose of 9 g in one patient, azathioprine in 2 patients, mycophenolate mofetil in 2 patients, cyclosporine A in four cases, hydroxychloroquine in all 6 previously treated cases, and thalidomide in 1 patient.

RTX was administered intravenously as previously described [11] at a dose of 375 mg/m² on days 2, 8, 15, and 22. Two more doses were administered 1 and 2 months following the last weekly infusion. This treatment was combined with two pulses of 10 mg/kg cyclophosphamide (days 4 and 17) and three intravenous pulses of 15 mg/kg (days 1, 4, and 8) methylprednisolone followed by oral prednisone, 0.8 mg/kg/day for 2 weeks rapidly tapered until 5 mg in 3 months.

Response was evaluated by assessing the changes in clinical signs and symptoms and laboratory parameters. SLEDAI score was separately assessed by two investigators (S.S. and M.A.).

Circulating B cells in the peripheral blood were investigated by detection of CD20+ B cells and analyzed by flow cytometry at baseline, month 1, month 2, and every other month thereafter.

We examined changes in T cell homeostasis following rituximab-induced B cell depletion in two patients. Analysis included flow cytometry studies at baseline (before the first RTX infusion), at months 3, 6, and 9. Whole blood samples obtained in the morning, in EDTA, were stained with monoclonal antibodies against CD45 (APC 100 eBioscience Bender Medsystems, CA, USA), CD3 (FITC eBioscience Bender Medsystems, CA, USA), CD4 (PC7 Beckman Coulter, CA, USA), CD19 (Pacific Blue™, Beckman Coulter, CA, USA), CD20 (PE Beckman Coulter, CA, USA), and CD25 (PerCP-eFluor 710 eBioscience/Bender Medsystems, CA, USA), FOXP3 (PE Staining set, eBioscience Bender Medsystems, CA, USA).

2.2. Renal response and relapse

For the evaluation of the renal response, the Joint European League Against Rheumatism and European Renal Association–European Dialysis and Transplant Association (EULAR/ERA-EDTA) consensus statement was used [13].

In detail, a complete renal response (CR) has been defined as proteinuria < 0.5 g/24 h and normal or near-normal (within 10% of normal GFR if previously abnormal) GFR and, additionally, negative anti-DNA antibodies and normal levels of C3 and C4. Partial response (PR) has been defined as ≥ 50% reduction in proteinuria to subnephrotic levels and normal or near-normal GFR.

The definition of renal flares included a reproducible increase of serum creatinine by ≥ 30% (or decrease in GFR by ≥ 10%) and/or an increase of proteinuria > 0.5 g/24 h if CR was initially achieved, or ≥ 50% in cases of PR.

2.3. Statistics

For comparison of variables at baseline and follow-up, Student's t-test was used for normally distributed parameters and the non-parametric Mann–Whitney test for non-normally distributed parameters. Correlations were calculated and significance determined by Fisher's test. Multivariable logistic regression analysis was used to identify any independent predictors of flare. Kaplan–Meier hazard plots were constructed for time to time to renal flare. For these analyses, with the Prism (GraphPad Software, CA, USA) and SPSS (IBM Corporation, NY, USA) software programs was used. $p < 0.05$ was considered significant.

This study was performed according to the local rules of off-label therapy in Piedmont Region (Northwest Italy).

3. Comparison with previously published literature

3.1. Selection of trials

We searched MEDLINE and EMBASE using the terms “rituximab” and “systemic lupus erythematosus” published from January 1, 2002, to May 24, 2015. Studies were included if they were randomized controlled studies or case series with more than 10 adult patients focused on renal clinical outcomes. Studies were excluded (1) if they were reviews or expert comments or case series with fewer than 10 patients or pediatric cases, (2) if the main outcome was not clinical, and (3) if they were published only in abstract form.

When several publications under the same group of patients were found, only the most recent and comprehensive paper was considered, unless the publication was derived from another patient cohort. Among the 651 sorted publications, 15 studies met the inclusion criteria, with 14 open-label trials on LN [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26] and [27] and one randomized controlled trial [28]. For each paper, selected items were systematically searched for: number of included patients,

follow-up, indication for treatment with RTX and dosage, concomitant immunosuppressive treatment, corticosteroid dosage, and clinical and biological outcomes.

4. Results

4.1. Our cohort

The mean duration of disease and follow-up after IBCDT was 13 years (range 3–24 years) and 44.5 months (range 24–93 months), respectively.

Following IBCDT, significant decreases ($p < 0.05$) were found in the ESR levels (baseline mean value: 55.0 mm; 3 months: 36 mm; end of follow-up: 13 mm), anti-dsDNA antibodies (baseline: 185 U; 3 months: 107 U; end of follow-up: 15 U), and proteinuria (baseline: 4.9 g/24 h; 3 months: 0.97 g/24 h; end of follow-up: 0.22 g/24 h). Conversely, C4 values (baseline 11 mg/dl) significantly increased ($p < 0.05$) after 3 months (22 mg/dl) and at the end of follow-up (20 mg/dl) (Fig. 1 and Fig. 2). Mean serum creatinine values were 0.89 mg/dl (0.6–2.6) at baseline, 0.87 (0.6–1.7) after 3 months and 0.83 mg/dl (0.6–1.1) at end of follow-up. IBCDT resulted in a decrease of median global SLEDAI from 16.5 [12], [13], [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26] and [27] to 4 [1], [2], [3], [4] and [5] at 12 months ($p < 0.001$) (Fig. 3A). Constitutional symptoms, including arthralgia, weakness, and fever, disappeared in all the previously affected patients, while serositis and skin lesions gradually resolved.

All patients had complete peripheral blood-B cell depletion. The CD20+ B cells were detectable in the circulation after a mean of 14.5 months (12–19 months) (Fig. 3B). Of note, at 36 months, CD20 cell number was still lower than baseline ($p < 0.01$).

Patients were not given any further immunosuppressive maintenance therapy during the follow-up, and oral prednisone was tapered to 5 mg/day by the end of the 3rd month after RTX.

Nine out of 12 patients (75%) remained in remission after one cycle of IBCDT, with no flare (mean 51.6 months [25–93]) (Fig. 4). Three patients relapsed after 36, 42, and 72 months, respectively. Following re-treatment, they again showed complete remission over 18–48 months of observation.

No serious adverse events were reported (2 cases of infusion speed-related bradycardia). Of note, no severe infections were observed during the follow-up.

In 5 patients achieving a CR within the first 6 months, a significant longer time to repopulation of B cells in the circulation was noted ($p = 0.05$) compared with the 7 patients who achieved CR later.

As shown in Fig. 5, upon detection of B cell depletion, we observed in 9 months a 3.5-fold increase the circulating Treg (CD4+CD25+FOXP3+).

5. Published literature

5.1. Open studies

Fourteen open-label trials [14], [15], [16], [17], [18], [19], [20], [21], [22], [23], [24], [25], [26] and [27] on patients with LN met our inclusion criteria (Table 1). All used renal biological values as criteria to assess clinical outcome with the definition of complete response (CR) and partial response (PR) detailed in Table 2. A more detailed analysis has been done on 4 prospective case series with more than 24 months of follow-up [18], [24], [25] and [26] (in bold in the Table 1). These trials included patients mostly with active LN despite treatment (World Health Organization or International Society of Nephrology/Renal Pathology Association class III (20 patients), IV (46 patients), III-V (4 patients), and IV-V (4 patients)). Thirty-three patients had class V LN. Patients received variable doses of RTX ($2 \times 1 \text{ g}$ $4 \times 375 \text{ mg/m}^2$), and immunosuppressant agents were continued, as detailed in Table 1.

5.2. Randomized controlled trial

The Lupus Nephritis Assessment with Rituximab (LUNAR) trial included patients with new or relapsed class III or IV (\pm class V) LN, supported by a renal biopsy [28]. Patients were randomized 1:1 to receive placebo or RTX 2×1 g repeated 6 months apart in conjunction with MMF and oral prednisone. Other immunosuppressive agents were stopped. Response criteria are detailed in Table 2. Among the 144 patients enrolled (72 patients in each group), global renal response rates (CR/PR) at week 52 were not statistically different between the RTX and the placebo groups (placebo group: CR 30.6%, PR 15.3%; and RTX group: CR 26.4%, PR 30.6%).

6. Discussion

The results of our study show that IBCDT can be a therapeutic option for patients with LN, with a favorable very long-term safety and efficacy profile. Seventy-five percent of patients remained in remission after one cycle of IBCDT without any further immunosuppressive maintenance treatment and did not relapse. These data confirm our previous results in case of refractory SLE [11]. The main interest of these findings resides in the relatively short time of standard immunosuppression, which strongly limits the possible adverse effects of steroids and cyclophosphamide assuring a long-lasting remission without immunosuppressive maintenance therapy [12]. Besides, this scheme might also be useful in low-compliance patients and avoid prolonged hospitalization.

When comparing to the available literature, we retrieved 4 open studies with clearly reported outcomes that included at least 10 patients and had a follow-up period of at least 24 months (mean 43.75 months). The overall response rate (including complete and partial responses) ranged from 72% to 90% (median 84%), the complete response rate from 61% to 72% (median 67%) (Fig. 6).

In our study, we observed an overall 100% of renal response albeit with a 25% rate of relapse meanly after 50 months of symptom-free period without urinary abnormalities (Fig. 6).

The following points should be considered.

First, in the mentioned studies [18], [24], [25] and [26], RTX has been used either as two doses of 1,000 mg given 2 weeks apart (two-dose regime, commonly used in RA) or as four doses of 375 mg/m² (four dose regime, most common regime used in lymphoma) given 1 week apart. Our scheme included two more doses administered 1 and 2 months following the last 375 mg/m² weekly infusion (so called "4 plus 2" scheme). This represents a novelty of this open single-center study and confirms our observations in other immune-mediated diseases [29], [30], [31], [32] and [33]. Notably, a systematic review of the clinical experience of RTX for the treatment of refractory SLE suggests that the lymphoma regimen may be more effective in achieving an improvement than the two doses regimen [34]. Besides, in our cohort, a delay in repopulation was found to increase the likelihood of achieving a CR within the 6 months. This observation would support the choice of a treatment-to-target approach to achieve an adequate degree of B cell depletion and a clinical response in LN. Several research groups have noted that the degree of B cell depletion is variable in SLE, but early repopulation is common in patients with a poor response to RTX [35]. The underlying reasons for the variability in B cell depletion remain elusive [7], [36], [37] and [38]. A polymorphism in Fc γ receptor IIIa has been shown to be important in achieving an adequate degree of B cell depletion, in favor of the high-affinity genotypes Fc γ receptor IIIa V158F (V, valine; F, phenylalanine) [39].

An IBCDT would therefore seem a rational approach, also taking into account that information derived from mouse models suggests that B cells exert their pathogenic role not only by producing auto-antibodies and regulating their specificities, but also by regulating the function of auto-reactive T cells, influencing antigen-presenting cells, and producing cytokines. Regulatory T cells (Treg) are critically involved in the

pathogenesis of autoimmune diseases by suppressing effector T cells proliferation and cytokine production [40] and [41]. Interestingly, Treg frequencies have been previously shown to increase in the blood of patients with SLE following RTX [42]. In our study, in a sub-cohort of IBCDT-treated patients with LN, we noticed that, at the onset of B cell depletion, patients displayed sharp increases in CD4+CD25+FOXP3+ cells in the peripheral blood. We hypothesized that a subset of these cells might represent expanded regulatory T cells (Treg), considered to be essential in the maintenance of peripheral self-tolerance [41]. This could be a secondary consequence of clinical remission, or, if Treg are actually associated with the disease process, these data might imply that Treg are in some way B cell dependent.

Besides, as previously emphasized [43] and [44], the possible synergistic effect of RTX in combination with cyclophosphamide could not be excluded in maintaining a sustained B cells depletion.

Second, albeit in our cohort a significant decrease of proteinuria and markers of activity has been observed since month 3, the time to CR was longer in most of the cases and documented within 12 months of follow-up. These data suggest that the efficacy of RTX has to be evaluated over longer follow-up periods in order to increase the likelihood of capturing complete responders in the differentiation between active treatment and comparators. This observation may be in line with the fact that the Lupus Nephritis Assessment with Rituximab (LUNAR) clinical trial, investigating the efficacy of RTX in patients with LN in a double-blind, randomized trial, failed to achieve its primary endpoint assessed at week 52, although the serological improvement was statistically better in the RTX group [28]. A further consideration is worthy of note in the contest of LUNAR. One-hundred-forty-four patients from 55 centers were enrolled, resulting in a mean of 2.62 patients for each center. Such heterogeneity might have significantly influenced the results of the trial. Third, a further interest of our regimen resides in the relatively short time of standard immunosuppression associated with a noticeable steroid sparing effect. Since the end of the third month of therapy, patients were given 5 mg prednisone. When compared to other studies with similar follow-up (bold references in Table 1), we observed a significantly lower rate of adverse events, especially in regard to severe infections (which did not occur in our cohort). The fact that RTX was not shown to be superior to conventional therapy in the LUNAR trial does not necessarily signify that it is inferior, mainly because its safety profile seems to be better than standard immunosuppression.

6.1. Limitations of the study

The main limitations of our study are the open non-blinded nature and the limited number of enrolled subjects. However, those are counterbalanced by the facts that our data are supported by a very long-term follow-up in a cohort of real-life SLE patients and that IBCDT allows a relatively short time of standard immunosuppression associated with a marked steroid sparing effect.

7. Conclusions

This prospective study brings additional long-term evidence of a role of RTX as an off-label agent in cases of LN who are intolerant or resistant to conventional therapy and need alternative therapeutic options or in patients with specific request of a short-time immunosuppression (e.g., with gestational perspectives). IBCDT obtained a long-lasting remission, which was maintained with minimal doses of prednisone by the end of the third month of therapy, avoiding the need of prolonged immunosuppression and minimizing the destructive effects of steroids. IBCDT could represent a valid alternative to offer patients treatments tailored to the individual needs based on the severity of the disease, previous medical history, or desire to have children and at same time minimizing the risk of adverse effects.

References

- [1] Yaniv G, Twig G, Shor DB, Furer A, Sherer Y, Mozes O, et al. A volcanic explosion of autoantibodies in systemic lupus erythematosus: a diversity of 180 different antibodies found in SLE patients. *Autoimmun Rev* 2015;14(1):75–9.
- [2] Kamal A, Khamashta M. The efficacy of novel B cell biologics as the future of SLE treatment: a review. *Autoimmun Rev* 2014;13(11):1094–101.
- [3] Gregersen JW, Jayne DR. B-Cell depletion in the treatment of lupus nephritis. *Nat Rev Nephrol* 2012;8(9):505–14.
- [4] Greco A, De Virgilio A, Rizzo MI, Gallo A, Magliulo G, Fusconi M, et al. Microscopic polyangiitis: advances in diagnostic and therapeutic approaches. *Autoimmun Rev* 2015;14(9):837–44.
- [5] Piatek CI, El-Hemaidi I, Feinstein DI, Liebman HA, Akhtari M. Management of immune-mediated cytopenias in pregnancy. *Autoimmun Rev* 2015;14:806–11.
- [6] Greco A, Rizzo MI, De Virgilio A, Gallo A, Fusconi M, Ruoppolo G, et al. Churg–Strauss syndrome. *Autoimmun Rev* 2015;14:341–8.
- [7] Ahmed AR, Shetty S. A comprehensive analysis of treatment outcomes in patients with pemphigus vulgaris treated with rituximab. *Autoimmun Rev* 2015; 14:323–31.
- [8] Reynaud Q, Durieu I, Dutertre M, Ledochowski S, Durupt S, Michallet AS, et al. Efficacy and safety of rituximab in auto-immune hemolytic anemia: a meta-analysis of 21 studies. *Autoimmun Rev* 2015;14:304–13.
- [9] Moulis G, Lapeyre-Mestre M, Montastruc JL, Sailler L. Exposure to non-corticosteroid treatments in adult primary immune thrombocytopenia before the chronic phase in the era of thrombopoietin receptor agonists in France. A nationwide populationbased study. *Autoimmun Rev* 2015;14:168–73.
- [10] Erkan D, Aguiar CL, Andrade D, Cohen H, Cuadrado MJ, Danowski A, et al. 14th International Congress on Antiphospholipid Antibodies: task force report on antiphospholipid syndrome treatment trends. *Autoimmun Rev* 2014;13:685–96.
- [11] Roccatello D, Sciascia S, Rossi D, Alpa M, Naretto C, Baldovino S, et al. Intensive shortterm treatment with rituximab, cyclophosphamide and methylprednisolone pulses induces remission in severe cases of SLE with nephritis and avoids further immunosuppressive maintenance therapy. *Nephrol Dial Transplant* 2011;26:3987–92.
- [12] Leah E. Therapy: Rituximab for refractory SLE—patients reach lasting remission with short-term regimen. *Nat Rev Rheumatol* 2011;7:312.
- [13] Bertias GK, Tektonidou M, Amoura Z, Aringer M, Bajema I, Berden JH, et al. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 2012;71: 1771–82.
- [14] Sfikakis PP, Boletis JN, Lionaki S, Vighlis V, Fragiadaki KG, Iniotaki A, et al. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. *Arthritis Rheum* 2005;52:501–13.
- [15] Vigna-Perez M, Hernandez-Castro B, Paredes-Saharopulos O, Portales-Perez D, Baranda L, Abud-Mendoza C, et al. Clinical and immunological effects of Rituximab in patients with lupus nephritis refractory to conventional therapy: a pilot study. *Arthritis Res Ther* 2006;8:R83.
- [16] Lindholm C, Borjesson-Asp K, Zendjanchi K, Sundqvist AC, Tarkowski A, Bokarewa M. Longterm clinical and immunological effects of anti-CD20 treatment in patients with refractory systemic lupus erythematosus. *J Rheumatol* 2008;35:826–33.
- [17] Melander C, Sallee M, Trolliet P, Candon S, Belenfant X, Daugas E, et al. Rituximab in severe lupus nephritis: early B-cell depletion affects long-term renal outcome. *Clin J Am Soc Nephrol* 2009;4:579–87.

- [18] Boletis JN, Marinaki S, Skalioti C, Lionaki SS, Iniotaki A, Sfikakis PP. Rituximab and mycophenolate mofetil for relapsing proliferative lupus nephritis: a long-term prospective study. *Nephrol Dial Transplant* 2009;24:2157–60.
- [19] Li EK, Tam LS, Zhu TY, Li M, Kwok CL, Li TK, et al. Is combination rituximab with cyclophosphamide better than rituximab alone in the treatment of lupus nephritis? *Rheumatology* 2009;48:892–8.
- [20] Pepper R, Griffith M, Kirwan C, Levy J, Taube D, Pusey C, et al. Rituximab is an effective treatment for lupus nephritis and allows a reduction in maintenance steroids. *Nephrol Dial Transplant* 2009;24:3717–23.
- [21] Garcia-Carrasco M, Mendoza-Pinto C, Sandoval-Cruz M, Soto-Vega E, Beltran-Castillo A, Jimenez-Hernandez M, et al. Anti-CD20 therapy in patients with refractory systemic lupus erythematosus: a longitudinal analysis of 52 Hispanic patients. *Lupus* 2010;19:213–9.
- [22] Ramos-Casals M, Garcia-Hernandez FJ, de Ramon E, Callejas JL, Martinez-Berriotxo A, Pallares L, et al. Off-label use of rituximab in 196 patients with severe, refractory systemic autoimmune diseases. *Clin Exp Rheumatol* 2010;28:468–76.
- [23] Catapano F, Chaudhry AN, Jones RB, Smith KG, Jayne DW. Long-term efficacy and safety of rituximab in refractory and relapsing systemic lupus erythematosus. *Nephrol Dial Transplant* 2010;25:3586–92.
- [24] Davies RJ, Sangle SR, Jordan NP, Aslam L, Lewis MJ, Wedgwood R, et al. Rituximab in the treatment of resistant lupus nephritis: therapy failure in rapidly progressive crescentic lupus nephritis. *Lupus* 2013;22:574–82.
- [25] Condon MB, Ashby D, Pepper RJ, Cook HT, Levy JB, Griffith M, et al. Prospective observational single-centre cohort study to evaluate the effectiveness of treating lupus nephritis with rituximab and mycophenolate mofetil but no oral steroids. *Ann Rheum Dis* 2013;72:1280–6.
- [26] Jonsdottir T, Zickert A, Sundelin B, Henriksson EW, van Vollenhoven RF, Gunnarsson I. Long-term follow-up in lupus nephritis patients treated with rituximab—clinical and histopathological response. *Rheumatology* 2013;52:847–55.
- [27] Moroni G, Raffiotta F, Trezzi B, Giglio E, Mezzina N, Del Papa N, et al. Rituximab vs mycophenolate and vs cyclophosphamide pulses for induction therapy of active lupus nephritis: a clinical observational study. *Rheumatology* 2014;53:1570–7.
- [28] Rovin BH, Furie R, Latinis K, Looney RJ, Fervenza FC, Sanchez-Guerrero J, et al. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum* 2012;64: 1215–26.
- [29] Roccatello D, Sciascia S, Rossi D, Alpa M, Naretto C, Russo A, et al. Long-term effects of rituximab added to cyclophosphamide in refractory patients with vasculitis. *Am J Nephrol* 2011;34:175–80.
- [30] Roccatello D, Baldovino S, Alpa M, Rossi D, Napoli F, Naretto C, et al. Effects of antiCD20 monoclonal antibody as a rescue treatment for ANCA-associated idiopathic systemic vasculitis with or without overt renal involvement. *Clin Exp Rheumatol* 2008;26:S67–71.
- [31] Cavallo R, Roccatello D, Menegatti E, Naretto C, Napoli F, Baldovino S. Rituximab in cryoglobulinemic peripheral neuropathy. *J Neurol* 2009;256:1076–82.
- [32] Roccatello D, Baldovino S, Rossi D, Mansouri M, Naretto C, Gennaro M, et al. Longterm effects of anti-CD20 monoclonal antibody treatment of cryoglobulinaemic glomerulonephritis. *Nephrol Dial Transplant* 2004;19:3054–61.
- [33] Sciascia S, Naretto C, Rossi D, Bazzan M, Roccatello D. Treatment-induced downregulation of antiphospholipid antibodies: effect of rituximab alone on clinical and laboratory features of antiphospholipid syndrome. *Lupus* 2011;20:1106–8.
- [34] Ramos-Casals M, Brito-Zeron P, Munoz S, Soto MJ, Group BS. A systematic review of the off-label use of biological therapies in systemic autoimmune diseases. *Medicine* 2008;87:345–64.

- [35] Vital EM, Dass S, Buch MH, Henshaw K, Pease CT, Martin MF, et al. B cell biomarkers of rituximab responses in systemic lupus erythematosus. *Arthritis Rheum* 2011;63: 3038–47.
- [36] Dumoitier N, Terrier B, London J, Lofek S, Mouthon L. Implication of B lymphocytes in the pathogenesis of ANCA-associated vasculitides. *Autoimmun Rev* 2015;14: 996–1004.
- [37] Visentini M, Tinelli C, Colantuono S, Monti M, Ludovisi S, Gagnani L, et al. Efficacy of low-dose rituximab for the treatment of mixed cryoglobulinemia vasculitis: Phase II clinical trial and systematic review. *Autoimmun Rev* 2015;14:889–96.
- [38] Berman H, Rodriguez-Pinto I, Cervera R, Morel N, Costedoat-Chalumeau N, Erkan D, et al. Rituximab use in the catastrophic antiphospholipid syndrome: descriptive analysis of the CAPS registry patients receiving rituximab. *Autoimmun Rev* 2013; 12:1085–90.
- [39] Anolik JH, Campbell D, Felgar RE, Young F, Sanz I, Rosenblatt J, et al. The relationship of Fcγ3R1 genotype to degree of B cell depletion by rituximab in the treatment of systemic lupus erythematosus. *Arthritis Rheum* 2003;48:455–9.
- [40] Sakaguchi S. Naturally arising Foxp3-expressing CD25 + CD4+ regulatory T cells in immunological tolerance to self and non-self. *Nat Immunol* 2005;6:345–52.
- [41] von Boehmer H. Mechanisms of suppression by suppressor T cells. *Nat Immunol* 2005;6:338–44.
- [42] Sfikakis PP, Souliotis VL, Fragiadaki KG, Moutsopoulos HM, Boletis JN, Theofilopoulos AN. Increased expression of the FoxP3 functional marker of regulatory T cells following B cell depletion with rituximab in patients with lupus nephritis. *Clin Immunol* 2007;123:66–73.
- [43] Ramos-Casals M, Diaz-Lagares C, Khamashta MA. Rituximab and lupus: good in real life, bad in controlled trials. Comment on the article by Lu et al. *Arthritis Rheum* 2009;61:1281–2.
- [44] Lu TY, Ng KP, Cambridge G, Leandro MJ, Edwards JC, Ehrenstein M, et al. A retrospective seven-year analysis of the use of B cell depletion therapy in systemic lupus erythematosus at University College London Hospital: the first fifty patients. *Arthritis Rheum* 2009;61:482–7.

Fig. 1.

Serologic profile of the SLE patients treated with In. Anti-DNA antibodies, erythrocytes sedimentation rate (ESR), and C4 were evaluated at 0, 3, 6, at 12 months and then yearly.

*p < 0.05; **p < 0.01.

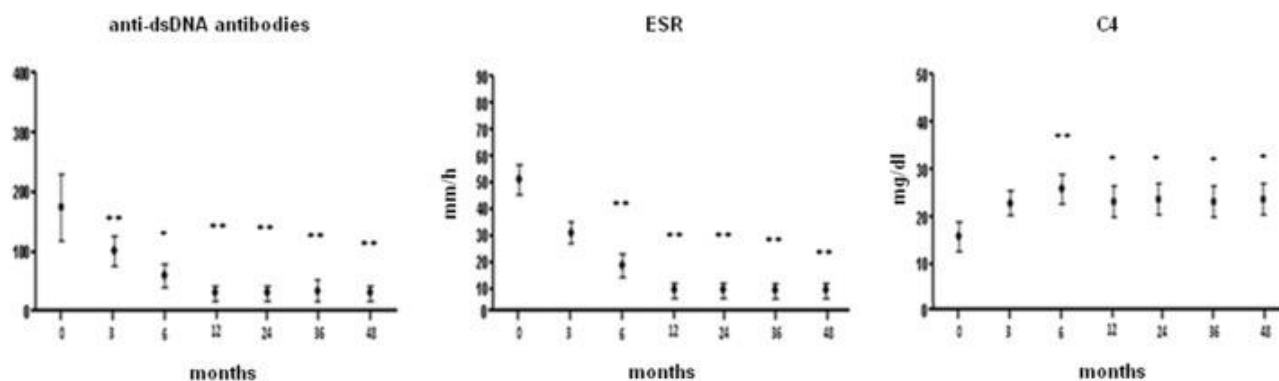


Fig. 2.

Proteinuria levels are shown. Proteinuria significantly improved since month 3 after intensified B cell depletion therapy protocol. Values are shown as mean \pm SEM. ***p < 0.001.

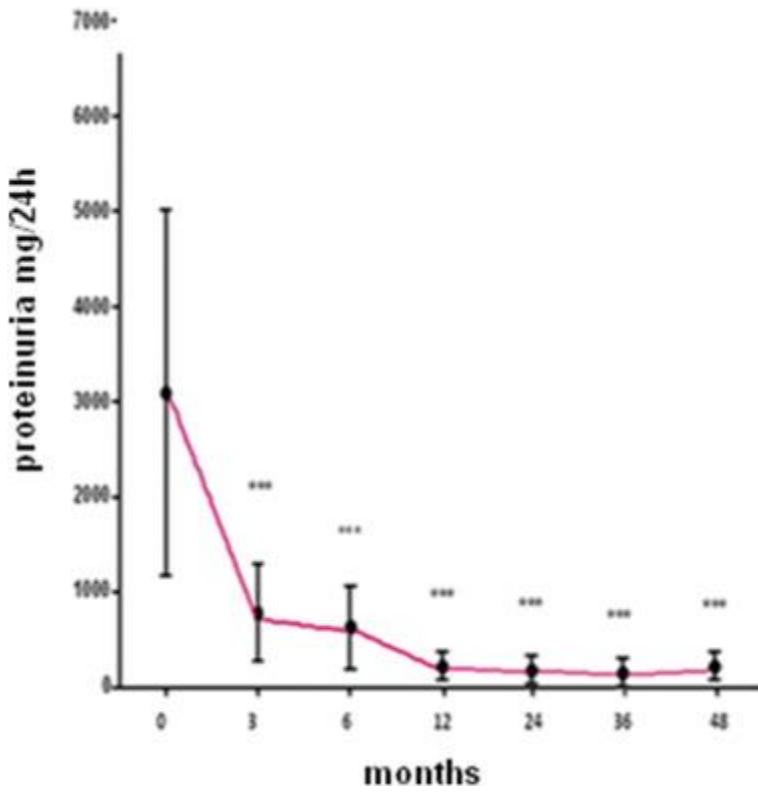


Fig. 3.

(A) SLEDAI values are shown at baseline and 12 months after intensified B cell depletion therapy protocol.
(B) Total numbers of CD20 + cells (B cells) in SLE patients before and after treatment with rituximab.

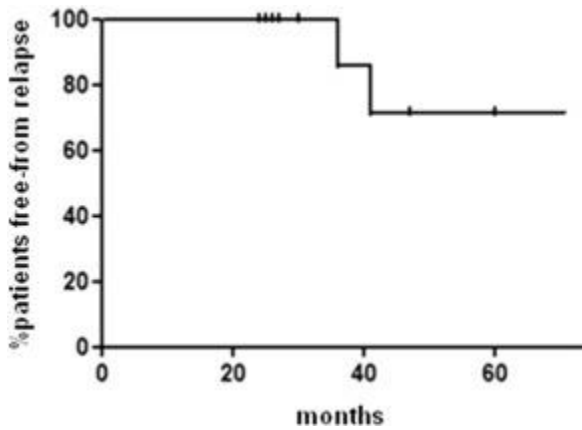


Fig. 5.

Representative dot-plots of Treg (CD4+CD25+FOXP3+, upper plots) and B cell (CD20+, lower plots) as evaluated by flow cytometry. Samples from a responder without relapse, were analyzed before and 3, 6, and 9 months after the intensified B cell depletion therapy.

At 1 month, upon detection of B cell depletion, the patient sample showed an increase of the percentage of Treg compared with baseline.

Of note, a 3.5-fold increase of the circulating Treg (CD4+CD25+FOXP3+) has been observed over 9 months.

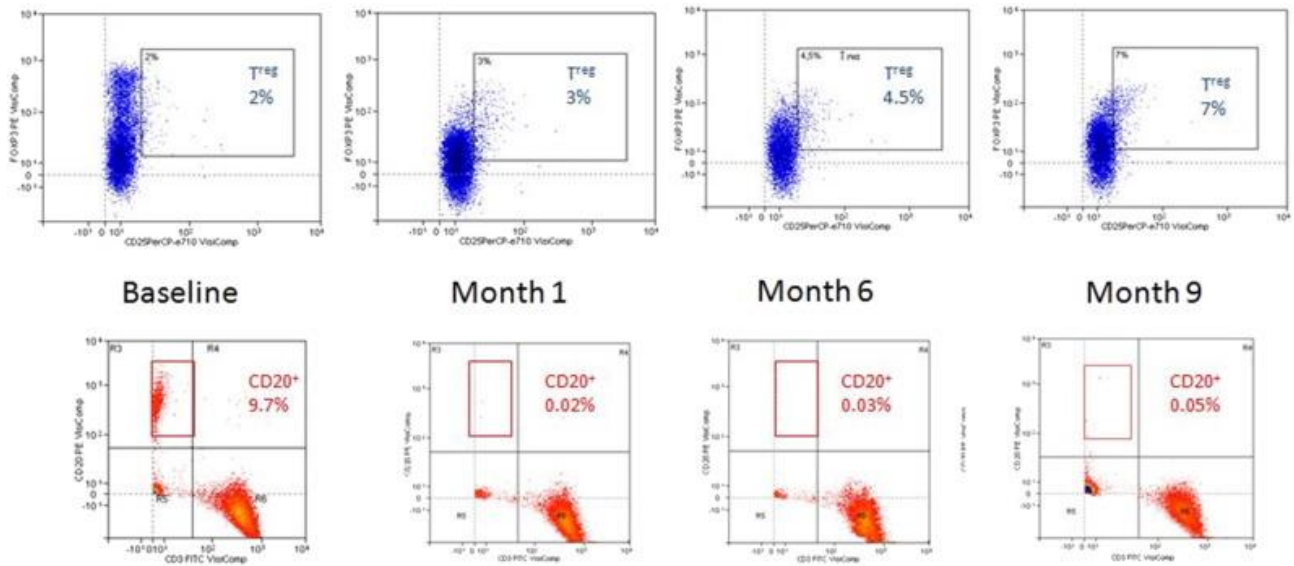


Fig. 6.

Rates of complete (CR) and partial responses (PR) and no response (NR) to RTX in patients with lupus nephritis. We retrieved 4 studies with clearly reported outcomes that included at least 10 patients and had a follow-up period of at least 24 months (mean 43.75 months). We compared these results to our data. The overall response rate (including complete and partial responses) ranged from 72% to 100% (median 88%), the complete response rate from 61% to 100% (median 70%). The flare rate (FR) ranged from 10% to 28% (median 24.4%). Renal responses are characterized as decreased proteinuria, improved kidney function, and decreased expression of serological markers of disease activity, in parallel with improved clinical measures.

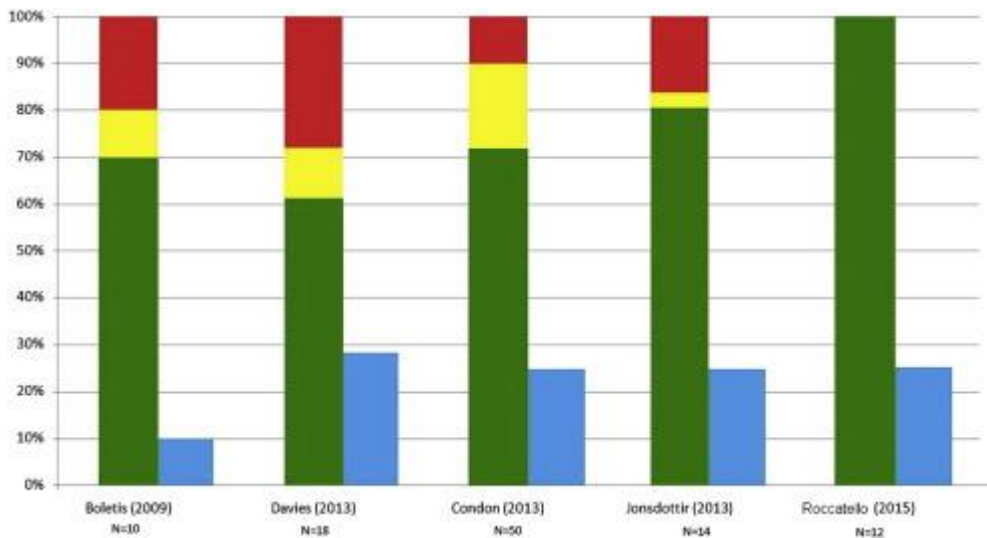


Table 1 Study characteristics and clinical outcome of rituximab-treated patients in lupus nephritis trials.

Study	Year	Design	N	RTX indication	RTX dose	IS continued	IS added	CG dose (mean)	F/U	Clinical outcome	Adverse event
Sfikakis	2005	PCS	10	Active LN (7 refractory, 3 induction)	4 × 375 mg/m ²	Oral GC	GC	0.5 mg/kg/day for 4 weeks then tapered	2-3	CR 50% PR 30%	NM
Vigna-Perez	2006	PCS	22	Refractory LN	2 × 0.5-1 g	RTX as add-on to previous treatment	None	NM	3	CR 23% PR 32%	1 infection
Lindholm	2008	RCS	17	Refractory LN	4 × 375 mg/m ²	RTX as add-on to previous treatment	None	NM	12	CR 12% PR 53%	1 infection, 1 serum sickness
Melander	2009	PCS	20	Active LN (18 refractory, 2 induction)	4 × 375 mg/m ²	RTX as add-on to previous treatment	CYC in 3 pts	0.7 mg/kg/day	22	CR 35% PR 25%	2 infections
Boletis	2009	PCS	10	Refractory LN	4 × 375 mg/m²	RTX as add-on to previous treatment	GC	0.5 mg/kg/day for 4 weeks then tapered	38	CR 70% PR 10%	NM
Li	2009	PCS	19	Active LN (6 induction)	2 × 1 g	None	GC and CYC	1 × 250 MTP then 30 mg/day PRED for 4 days then 0.5 mg/kg/day for 4 weeks then tapered	12	CR 21% PR 58%	2/3 infections
Pepper	2009	RCS	18	Active LN (6 induction)	2 × 1 g	RTX as add-on to previous treatment but CYC	MTP 2 × 500 mg (in 8 pts) and MMP 1 g	10 mg/day at entrance	12	CR 33% PR 33%	1 infection, 2 serum sickness
Garcia-Carrasco	2010	RCS	13	Refractory LN	2 × 1 g	RTX as add-on to previous treatment	MTP 2 × 500 mg	16 mg/day at entrance	6	CR 38% PR 38%	4 infections, 2 infusion reactions
Ramos-Casals	2010	RCS	49	Refractory LN	4 × 375 mg/m ² or 2 × 1 g	RTX as add-on to previous treatment	NM	NM	0-26	CR 80%	11 infections, 7 infusion reactions
Catapano	2010	RCS	11	Refractory LN	4 × 375 mg/m ² or 2 × 1 g	RTX as add-on to previous treatment	Heterogeneous**	10 mg/day at entrance	4	CR 36% PR 55%	NM
Rovin	2012	RCT	144	Active LN	4 × 1 g or placebo	None	1.5-3 g OD MMP, 2X 1000 mg MTP and then tapered GC	0.75 mg/kg/day then tapered	12	CR 26% PR 30%- - CR 30% PR 15%	
Davies	2013	PCS	18	Refractory LN	2 × 1 g	None	MTP 2 × 500 mg CYC 2 × 500 mg	NM	Mean 60	CR 61% PR 11%	1 infection, 7 infusion reactions
Condon	2013	PCS	50	Active LN	2 × 1 g	None	MMP based on MPA level (up to 1.5 g BD) CYC 2 × 0.5 g (2 patients MMP and 2 RTX monotherapy)	None	Mean 41 (13-60)	CR 72% PR 18%	5 infections
Jonsdottir	2013	PCS	14	Active LN (2 induction)	4 × 375 mg/m² or 2 × 1 g	None (1 patient MMP)	CYC 2 × 0.5 g (2 patients MMP and 2 RTX monotherapy)	0.5 mg/kg/day then tapered	Mean 36 weeks (9-95)	CR 64% PR 24%	6 infections, 3 infusion reactions (at 1 year)
Moroni	2014	PCS	17	Active LN (3 refractory)	2 × 1 g	None	MTP 3 × 500-1000 mg then GC, then AZA or MMP or Cys	0.5-0.75 (1) mg/kg/day for 4 weeks then tapered.	12	CR 70% PR 29%	2 infections, 1 infusion reaction
Roccatello	2015	PCS	12	Active LN (6 induction in naive patients)	4 × 375 mg/m² weekly + 2 further infusions after 1 and 2 months	None	CYC 2 × (10 mg/kg) and 3 MTP × 3 (15 mg/kg)	0.8 mg/die, rapidly tapered to 5 mg/day by the end of the 3rd month after RTX	mean of 44.5 (24-93) months	CR 100%	2 infusion speed-related bradycardia, 4 UTI

Studies with more than 24 months of follow-up are marked in bold.

Table 2 Definitions of complete and partial responses in lupus nephritis trials

Study	Complete response				Partial response		
	Proteinuria/24 h	Creatinine	Urinary sediment (microscopic evaluation)	Albumin	Proteinuria/24 h	Creatinine	Urinary sediment (microscopic evaluation)
-	<0.5 g	Normal	Inactive	Normal	Improvement ≥50% of previously altered parameters		
Sfikakis	<0.5 g	Normal	Inactive	-	Improvement ≥50% of previously altered parameters		
Vigna-Perez	<0.5 g	Normal	Inactive	-	Reduction ≥50%	Stable/normal	
Lindholm	<0.5 g	Normal or improved	Absence of hematuria	Normal	Reduction ≥50%	Stable/normal	-
Melander	<0.5 g	Normal	Inactive	Normal	Improvement ≥50% of previously altered parameters		
Boletis	<0.5 g	NR	RBC/HPF <10 WBC/HPF <10	NR	Reduction ≥50%	Stable/normal	-
Li	<0.5 g	NR	RBC/HPF <10 WBC/HPF <10	NR	Reduction ≥50%	Stable/normal	-
Pepper	<0.5 g	Normal	Inactive	Normal	Reduction ≥50%	Stable/normal	NR
Garcia-Carrasco	<0.5 g	Normal	Inactive	Normal	Improvement ≥50% of previously altered parameters		
Ramos-Casals	<0.5 g	Normal	Inactive	Normal	Reduction ≥50%	Stable/normal	NR
Catapano	<0.5 g	Normal	Inactive	NR	Reduction ≥50%	Stable/normal	RBC/HPF <30
Rovin	UPCR ratio <0.5	Normal	Inactive	NR	Reduction ≥50%(UPEC)	No greater than 15% above baseline	Reduction RBC/HPF >50%, with no cylinders
Davies	<0.5 g	Normal	Inactive	Normal	Improvement ≥50% of previously altered parameters		
Condon	UPCR <50 mg/mmol	<15% above baseline	NR	NR	<3.5 g with Reduction of UPCR ≥50%	No greater than 15% above baseline	NR
Jonsdottir	<0.5 g	Normal	Inactive	Normal	Reduction ≥50%	Stable/normal	NR
Moroni	<0.5 g	Normal or <1.2 mg/dL	RBC/HPF <5	NR	0.5-2 g	Normal or <1.2 mg/dL	NR
Roccatello ^a	<0.5 g	Normal	Inactive	Normal	Reduction ≥50%	Stable/normal	NR

^a Additionally, CR definition includes negative anti-DNA antibodies and normal levels of C3 and C4.