First line use of Rituximab versus standard corticosteroid regimen in the treatment of patients with Pemphigus: a multicenter randomized study

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Summary

Background: High corticosteroid (CS) doses are considered the standard treatment for pemphigus. We evaluated whether first-line use of rituximab could improve the rate of complete remission (CR) off-therapy, while decreasing CS cumulative doses and treatment-side-effects.

Methods: Patients with newly diagnosed pemphigus were randomly assigned to receive a standard-CS regimen using 1 or 1.5 mg/kg/day of prednisone tapered over 12 or 18 months, or 1000 mg of rituximab on days 0 and 14, and 500 mg at months 12 and 18, associated with 0.5 or 1 mg/kg/day of prednisone tapered over 3 or 6 months. The primary end-point was CR-off-therapy at month 24.

Results: Ninety patients were randomized. At month 24, 41 of 46 patients (89%) assigned to the rituximab group were in CR-off-therapy versus 15 of 44 (34%) in the standard-CS group (absolute difference, 55 percentage points; 95% confidence interval (95%CI; 38.4-71.7; P<0.0001), corresponding to 1.82 (95%CI=1.39-2.60) patients needing to be treated with rituximab for an additional success. Median (range) cumulative duration of CR-off-therapy was over 7-fold higher in patients assigned to the rituximab group, 446 days (0-567 days), than to the standard-CS group, 62 days (0-608 days), (P<0.0001). Patients in the rituximab group took about one-third the prednisone cumulative dose of prednisone, (mean±standard deviation), 6,143±2,411mg relative to the standard-CS group, 17,973±7,272mg, (P<0.0001), and experienced about half as many severe adverse-events, 0.50±1.13 versus 0.93±1.17 per patient, (P=0.0084).

Conclusion: First-line use of rituximab in pemphigus is more effective than standard-prednisone regimen, while allowing a major CS-sparing effect, and fewer adverse events.

Introduction

Pemphigus is a life-threatening autoimmune blistering disease affecting the skin and mucosa (1-5). It is mediated by pathogenic autoantibodies directed against desmoglein 1 and desmoglein 3 adhesion molecules of the epidermis, that are responsible for the cohesion between keratinocytes in skin and mucosa (6-8). High doses of systemic corticosteroids (CS) are considered the standard treatment for patients with pemphigus (9,10). There is only limited evidence to support the use of conventional immunosuppressants as first line treatment of pemphigus (11-19).

Rituximab, a monoclonal antibody directed against the CD20 antigen of B lymphocytes, has been demonstrated to be highly effective in severe types of pemphigus (20-31).Some uncontrolled case-series have suggested that the use of rituximab as first line treatment of pemphigus permits rapid tapering of CS doses (21, 32). Because long-term CS treatment is responsible for severe and even life-threatening side effects in patients with pemphigus (33-35), we conducted a randomized trial comparing a "standard" treatment with high dose prednisone given for 12 to18 months, and a regimen associating rituximab and lower initial doses of prednisone, rapidly tapered over 3 to 6 months. The aim of this study was to assess whether this ''rituximab + mild CS regimen" could substantially improve the rate of long-term complete remission (CR) off-therapy in patients with pemphigus, whilst allowing a decrease in cumulative doses of prednisone and a reduction of severe treatment adverse events, compared to the "standard" CS-regimen.

Methods

Study Oversight

This trial, Ritux 3, was designed by the principal investigator (P.J.) who also drafted and wrote the manuscript. The Ethics Committee (CPP Nord-Ouest1) approved the study. Roche provided rituximab for the study, but had no role in designing, conducting,

analyzing the study or writing this manuscript. The references of the trial (ClinicalTrials.gov number, NCT00784589) and full protocol are available in the supplementary Appendix.

Patients

Consecutive patients between 18 and 80 years of age with newly diagnosed pemphigus were eligible when they fulfilled the following criteria: i) clinical features suggestive of pemphigus vulgaris (PV) or pemphigus foliaceus (PF); ii) a histologic image of intra-epidermal acantholysis; and iii) deposition of IgG, complement component 3, or both on the keratinocyte membrane detected by direct immunofluorescence (13). Exclusion criteria are listed in the Supplementary Appendix. Classification of pemphigus severity was performed based on Harman's criteria (36), since no threshold differentiating moderate from severe pemphigus was available at the time the study was designed, whether for the Pemphigus Disease Area Index (PDAI) or Autoimmune Bullous Skin Disorder Intensity Score (ABSIS) scores (37,38). Severe pemphigus was defined as skin involvement of more than 5% of the body surface area, and/or significant mucosal involvement defined as more than 10 mucosal erosions, or diffuse gingivitis or confluent large erosions, and/or involvement of two or more mucosal sites. All patients provided written informed consent.

Treatment Protocol

This randomized study compared two parallel groups of patients treated either by a standard-CS regimen using high initial doses of prednisone which were tapered over 12 to 18 months, or rituximab associated with a mild-CS regimen using reduced initial doses of prednisone with rapid tapering over 3 to 6 months. Since the prednisone doses and their tapering were different between the 2 arms, the study was not blinded. Treatment

was assigned through central computer-generated randomization, with stratification on disease-severity (severe, moderate).

Patients in the rituximab group received intravenous rituximab at a fixed 1000-mg dose on days 1 and 14 following randomization, and then 500 mg at months 12 and 18. In addition, they took oral prednisone (Cortancyl®) once a day at an initial dosage of 0.5 mg per kilogram per day for moderate pemphigus and 1 mg per kilogram per day for severe pemphigus. The initial prednisone dose was maintained for 1 month and thereafter gradually reduced after achievement of disease control, with the aim to stop prednisone after 3 months in patients with moderate pemphigus and after 6 months in patients with severe pemphigus. Disease control was defined as the time at which new lesions ceased to form and established lesions began to heal (39).

Patients randomly assigned to the standard-CS group received an initial dose of prednisone (Cortancyl®) of 1 mg per kilogram per day for moderate pemphigus and 1.5 mg per kilogram per day for severe pemphigus. The initial dose of prednisone was maintained for 1 month, and thereafter gradually tapered in patients who achieved disease control, in order to stop prednisone after 12 months in patients with moderate pemphigus, and after 18 months in patients with severe pemphigus. The protocol for tapering prednisone doses and for treatment of relapses is detailed in the Supplementary Appendix. During the entire study period, no other therapy expected to be active on pemphigus was allowed. In both treatment groups, investigators were allowed to stop treatment if a very severe or life-threatening side effect occurred, or if a flare of pemphigus was not controlled despite the successive incremental increases in prednisone doses as scheduled in the protocol.

Follow-up was initially planned for two years but, in order to assess the rate of relapse after the last infusion of rituximab at month 18, the follow-up was extended to 3 years.

Study Assessments

Study visits were scheduled weekly during the first month of treatment and then monthly until month 24. An additional visit was subsequently scheduled at month 36.

At each study visit, prednisone dose as well as PDAI, ABSIS and Physician Global Assessment (PGA) pemphigus severity scores (see Supplementary Appendix for their description) were recorded (37,38). The Dermatology Quality of Life Index (DLQI) and Skindex quality of life scores were recorded at baseline and months 3, 6, 9,12, 18 and 24 (39,40). Blood samples were collected at each study visit. Serum anti-desmoglein 1 and 3 antibodies, blood CD19+ B-lymphocytes and desmoglein- specific B lymphocytes were analyzed as detailed in the supplementary Appendix.

Endpoints

The primary endpoint was CR off-therapy at month 24, defined as the absence of new or established lesions while the patient had been off-CS for at least two months (41). Secondary endpoints were: i) CR on-minimal therapy at month 24 (41); ii) delay to achievement of CR off-therapy; iii) cumulative duration of CR off-therapy during the study; iv) relapse occurrence; v) cumulative dose of prednisone during the study; vi) time change of DLQI and Skindex quality of life scores; vii) occurrence of severe treatment adverse events, (grade 3 or 4, or death of any cause) (42).

Statistical Analyses

From our preliminary studies and the literature (1,14,15,21), we hypothesized that the rates of patients in CR off-therapy at month 24 would increase from 40% with standard-CS therapy to 70% with rituximab. To achieve 80% power relative to this difference for Pearson's chi-square test at the two-sided 0.05 level and allowing for 5% dropouts, 90 patients (2x45) were required for enrolment. Analyses were based on the intention-to-

treat principle. Patients who withdrew from the study were considered as not having reached CR off-therapy at month 24. Proportions of patients achieving CR off-therapy at month 24 were compared between the two treatment groups using Pearson's chi-squared test. Absolute rate difference and number needed to treat (NNT) were estimated along with corresponding 95% confidence intervals (CIs). This crude comparison was complemented by a comparison adjusting for gender or PDAI score based on the log-binomial regression model (PROC GENMOD) with the COPY method (43), from which adjusted relative risks (RR) of CR off-therapy at month 24 and their 95% CI were estimated. We adjusted for the PDAI rather than the ABSIS or PGA scores, since it results from an international consensus and is the only validated score (38).

Statistical methods used to analyze secondary endpoints are detailed in the Supplementary Appendix.

All statistical tests used the two-sided 0.05 level as their significance threshold. For quantitative variables, mean ± standard deviation or median (range) were reported. All analyses were performed with software SAS (version 9.3, SAS Institute, Cary, NC).

Results

Patients

Between May 2010 and December 2012, 91 patients from 25 centers in France were enrolled in the study but one patient withdrew his consent before randomization, leaving 90 randomized patients overall (46 in the rituximab group and 44 in the standard-CS group) (Fig 1 supplementary appendix). Seventy-four out of 90 patients (82%) had PV. Mean weight loss before treatment in the 71 patients who had oral lesions was 6.7±4.5 kg. Clinical characteristics of patients according to treatment groups are shown in Table 1. The two groups were well balanced except for gender, and PDAI score, which was higher in patients assigned to the standard CS group (46.0±23.7 points on a scale ranging

from 0 to 250 points) than to the rituximab group (33.5±28.1 points, P=0.0275). The median follow-up of all study patients was 729 days (interquartile range: 711-744 days) and that of patients who did not withdraw from the study was 733 days (interquartile range: 727-749 days).

Study Endpoints

Complete remission off-therapy

At month 24, 41 of 46 patients (89%) were in CR off-therapy in the rituximab group versus 15 of 44 patients (34%) in the standard-CS group (absolute difference, 55 percentage points; 95% CI, 38.4 -71.7; P<0.0001). This difference corresponded to a relative risk (RR) of success of 2.61 (95% CI, 1.71-3.99; P<0.0001) and translated to a NNT of 1.82 patients (95% CI, 1.39-2.60), i.e., 1.82 patients on average would need to be treated with rituximab rather than standard-CS in order to obtain one additional success. After adjusting for gender or baseline PDAI score, a strong beneficial effect of rituximab was still evidenced with RR of CR-off therapy of 2.66 (95% CI, 1.73-4.07; P<0.0001) and 2.55 (95% CI, 1.41-3.69; P<0.0001), respectively. When only considering the subgroup of patients with PV, 34 of 38 patients (89%) in the rituximab group and 10 of 36 patients (28%) in the standard-CS group were in CR off-therapy at month 24, (absolute difference 61.7 percentage points; 95% CI, 44.1-79.3; P<0.0001). The NNT was 1.62 (95% CI, 1.26-2.27), unadjusted RR was 3.22 (95% CI, 1.88-5.52; P<0.0001), RR adjusted for gender was 3.24 (95% CI, 1.89-5.57; P<0.0001), and RR adjusted for PDAI score was 3.00 (95% CI, 1.38-4.63; P<0.0001).

Secondary endpoints

Complete remission on-minimal therapy

At month 24, no patient in the rituximab group and one patient in the standard-CS group were both in CR and still receiving minimal therapy. Five patients in the rituximab group

and 28 patients in the standard-CS group still had active lesions at month 24, or had no lesions but still took a prednisone dose higher than 10 mg/day.

Cumulative duration of CR off-therapy during the study

Median delay to achieve CR off-therapy was 677 days (range: 420 -713 days) in the standard-CS group and 277 days (range: 177-751 days), in the rituximab group (P<0.0001). Kaplan-Meier estimates showed that patients in the rituximab group had a higher cumulative probability of achieving CR off-therapy during the study than patients in the standard-CS group (HR: 7.75; 95% CI: 4.27-14.08; P<0.0001) (Fig 1). Median cumulative duration of CR off-therapy during the study was over 7-fold higher in patients assigned to the rituximab group, 446 days (range: 0-567 days), than in the standard-CS group, 62 days (range: 0-608 days; P<0.0001).

Relapses

At month 24, relapse had occurred in 11 patients in the rituximab group and 20 patients in the standard-CS group. Kaplan-Meier disease-free survival curves showed a higher relapse rate in the standard-CS group than in the rituximab group, with 2-year disease-free survival rates of 36.7%, (95%CI, 14.5-59.5%) and 75.4% (95%CI: 59.9-85.5%), respectively (P=0.0191) (Fig.2). During the third year of follow-up, a relapse occurred in 1 of the 41 patients who were in CR off therapy at month 24 in the rituximab group (2%) and in 4 of the 15 patients in the standard-CS group (27%).

Cumulative dose of prednisone and Quality of life

Patients assigned to the rituximab group took one-third the cumulative dose of prednisone during the study, 6,143.1 ± 2,411.5 mg, relative to patients in the standard-CS group, 17,973.6 ± 7 272.5 mg, (P<0.0001). The DLQI and Skindex scores showed greater improvements in patients in the rituximab group than in the standard-CS group (P=0.0411, and P=0.0137, respectively).

Death and treatment adverse events

No patient died during the study. Fourteen patients withdrew from the study; 2 patients in the rituximab group (pregnancy, n=1; treatment failure, n=1), and 12 in the standard-CS group (treatment failure, n=4; treatment adverse-events, n=8, including severe myopathy, n=2; septic arthritis, n=1; hip osteonecrosis, n=1; psychosis, n=1; chorioretinitis, n=1; 25 kg weight gain, n=1; cardiac failure, n=1). Overall, 53 severe adverse-events were reported in 29 patients from the standard-CS group (1.20±1.25 per patient), and 27 in 16 patients from the rituximab group (0.59±1.15 per patient; P=0.0021) (Table 2).

Anti-desmoglein- B-cell response

Figure 3 shows a dramatic and long-lasting disappearance of total and desmoglein-specific B-lymphocytes in patients from the rituximab group, whereas they remained unchanged until the end of the study in patients from the standard-CS group. Accordingly, anti-desmoglein antibodies which initially decreased from treatment initiation to month 12 in both treatment groups, later re-increased from month 12 to month 24 in patients treated with the standard-CS regimen, whereas they remained at very low levels in patients from the rituximab group. This was also in accordance with the occurrence of 13 relapses in patients from the standard-CS group between months 12 and 24 when prednisone doses were tapered under 20 mg/day, whereas only 3 relapses were observed during this time period in patients treated with rituximab.

Discussion

This study demonstrates that first line use of rituximab associated with a mild-CS regimen was superior to a standard-CS regimen on all primary and secondary end points. This was true not only for pemphigus patients as a whole, but also in patients with PV, which is often considered to be a more severe condition than PF. High doses of

prednisone were chosen as the standard-regimen, since all RCT in the literature failed to demonstrate a clear beneficial effect from the addition of conventional immunosuppressants to CS alone (11-19). In addition, most of these studies reported a high rate of treatment-adverse events in patients with the combined treatment. Initial treatment with rituximab resulted in a 2.5-fold increase in the rate of achievement of CR-off therapy at month 24 compared to a standard-prednisone regimen. This strong beneficial effect of rituximab was still apparent after adjusting for the gender and baseline PDAI score, since the relative risk of CR-off therapy did not vary significantly after adjustment. Importantly, rituximab permitted the rapid tapering of CS doses, since about 60% of patients were able to stop prednisone after 6 months of treatment, resulting in a 7-fold higher duration of CR-off therapy during the study, than with the standard-CS regimen. Additionally, patients in the rituximab group had a lower rate of severe adverse-events than those in the standard-CS group, which was likely due to the use of a 3-fold lower cumulative dose of prednisone during the study.

The present trial has some limitations. First, it was not blinded, since the tapering of prednisone doses was different in the 2 treatment groups. Indeed, the same rapid tapering of prednisone in both treatment groups would have allowed the use of a placebo for rituximab in the standard-CS group, but would have induced an unethical and artificially high level of relapses in this group, not corresponding to the standard of care for pemphigus patients. Additionally, the use of a placebo for rituximab with 2 different regimens of CS tapering would have been impractical in such a multicenter study.

Second, one out of the four scoring systems used, the PDAI score was slightly higher in patients from the corticosteroid only group than in those receiving rituximab + corticosteroid. However, we believe that it is highly unlikely that this small intergroup difference corresponding to about 5% of the scale of this scoring system could constitute a bias that might explain the major therapeutic effect observed in this study. This is also strongly suggested by the fact that the relative risks of treatment success were almost the

same after adjusting for the baseline PDAI score, irrespective of the population considered, all pemphigus patients or patients with pemphigus vulgaris. Third, despite the fact that the primary endpoint was assessed after a rather long 2-year follow-up time, one could argue that this assessment was close to the last maintenance infusion of rituximab at month 18. This is why we prolonged the follow-up of patients to month 36, which showed an extremely low (2%) rate of relapse of patients after the last infusion of rituximab, suggesting a long-lasting beneficial effect of these maintenance infusions. Finally, the 500 mg dose of rituximab used for maintenance, and the time intervals between infusions were somewhat arbitrary, although rather similar to those used in ANCA associated vasculitis (44). The occurrence of eight out of the eleven relapses in the rituximab group from month 6 to month 12, as well as a re increase of total and desmoglein-specific blood B-lymphocytes and circulating anti-desmoglein antibodies during this time interval suggest that the first maintenance infusion of rituximab might have been of more value if performed at month 6 instead of month 12.

We observed that the dramatic improvement in the patients' condition in the rituximab group paralleled the long-lasting disappearance of desmoglein-specific circulating B-lymphocytes and consequently, anti-desmoglein antibodies. These findings strongly contrast with the persistence of desmoglein-specific B-lymphocytes in patients from the standard-CS group and the subsequent increase of anti-desmoglein antibodies from month 12 when prednisone doses were tapered under 20 mg/day, which was likely responsible for the high relapse rate of patients in the standard-CS group. These findings also contrast with the re increase of anti-desmoglein antibodies after month 6 that we observed in our initial study, in which patients were treated with a single cycle of rituximab without maintenance infusions (30). They provide a biological rationale for maintenance infusions of rituximab to maintain a prolonged failure of anti-desmoglein antibodies to minimize the occurrence of relapse after the initial cycle.

In conclusion, the between-group differences in this trial strongly argue for first line use of rituximab in patients with pemphigus. It is both more effective and safer, allowing rapid tapering of CS and causing fewer treatment adverse events, which is likely related to the profound and long-lasting disappearance of anti-desmoglein B-cell response.

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Table 1: Baseline characteristics of study patients according to assigned treatment group

	Standard Corticosteroid (n = 44)		**Rituximab + mild Corticosteroid** (n = 46)		**P**
Age (years)					
Mean ± SD	53.1 ± 13.8		53.5 ± 16.2		0.9144
Gender - no. (%)					
Female	19	(43.2)	31	(67.4)	0.0209
Male	25	(56.8)	15	(32.6)	
Pemphigus subtype - no. (%)					
Vulgaris	36	(81.8)	38	(82.6)	0.9219
Foliaceus	8	(18.2)	8	(17.4)	
Severity of pemphigus					
Harman's criteria no.(%)					
Moderate	5	(11.4)	6	(13.0)	0.8078
Severe	39	(88.6)	40	(87.0)	
ABSIS * mean ± SD	43.6 ± 24.1		34.4 ± 20.6		0.0614
PDAI ** mean ± SD	46.0 ± 23.7		33.5 ± 28.1		0.0275
PGA *** mean ± SD	6.9 ± 1.4		6.4 ± 1.6		0.0977
Quality of life					
SKINDEX° mean ± SD	60.3 ± 23.7		54.4 ± 24.3		0.3149
DLQI°° mean ± SD	11.6 ± 7.0		10.2 ± 6.4		0.3473

* standard CS regimen group: n= 40; rituximab group: n=45 ; ** standard CS regimen group: n= 42; rituximab group: n=46 ; *** standard CS regimen group: n= 42; rituximab group: n=46; ° standard CS regimen group: n= 32; rituximab group: n=38; °° standard CS regimen group: n= 38; rituximab group: n=44. P: from Pearson chi-square test for categorical variables and Student t-test for continuous variables

Table 2: Number and type of grade 3-4 severe treatment side effects according to assigned treatment group

Type of treatment adverse event	**Standard Corticosteroid (n=53)**	**Rituximab + mild Corticosteroid (n=27)**
	Number	**Number**
Diabetes mellitus*, endocrine disorders	11	6
Major weight gain (>10Kg)	4	0
Severe myopathy	10	3
Cardiovascular disorders**	7	3
Bone disorders***	5	5
Severe infections, excluding pneumonia	4	2
Pneumonia	1	3
Psychiatric disorders	4	2
Neurological disorders	3	0
Skin / ENT disorders	1	2
Ocular disorders	2	0
Electrolyte disorder	1	0
Hepatitis	0	1

* Only cases of diabetes requiring insulin were included in the analysis.
** Myocardial infarction, cardiac failure or pulmonary embolism
*** Data include both clinically apparent fractures and vertebral fractures visible on radiography.
The detailed distribution of treatment adverse events is shown in the supplementary appendix

Legend to Figures

Figure 1: Cumulative probability of achieving CR-off therapy.

Kaplan-Meier estimates of the cumulative probability of achieving a first episode of complete remission off-therapy during the study (red line: patients treated with the standard corticosteroid regimen; blue line: patients treated with rituximab). The hazard ratio for achieving CR off therapy in patients from the rituximab group compared to patients in the standard corticosteroid group, was 7.75, (95% CI: 4.27-14.08; P<0.0001)

Figure 2: **Clinical and biological response to treatment.**

Kaplan-Meier estimates of disease-free survival (panel A); evolution of prednisone doses (mg/day) (panel B); and anti-desmoglein 1 and anti-desmoglein 3 antibody ELISA values (panels C and D) of pemphigus patients according to treatment regimen (red line: patients treated with the standard corticosteroid regimen; blue line: patients treated with rituximab). Arrows indicate when rituximab infusions were performed.

Patients were randomly assigned to receive rituximab (1000 mg on days 1 and 14, and 500 mg at months 12 and 18 after the first infusion (arrows), associated with an initial dose of 0.5 mg to 1mg per kilogram per day oral prednisone with a tapering of prednisone doses over 3 to 6 months, or an initial dose of prednisone of 1 mg to 1.5 mg per kilogram per day with a tapering of prednisone dose over 12 to 18 months.

Panel A shows the probability of remaining free of relapse after randomization. The hazard ratio for relapse for patients in the standard corticosteroid group, compared to patients in the rituximab group, was 2.35 (95% CI, 1.12-4.92; P = 0.0191).

*10 relapse-free patients in the standard CS group and 12 in the rituximab group had their month 24 visit at a mean time of 14.8 ± 11.5 days and 13.6 ± 9.5 days before day 730.

Panel B shows the tapering of prednisone doses during the study period. Patients assigned to the rituximab group took a 3-fold lower cumulative dose of prednisone during the study, 6,143.1 ± 2,411.5 mg, than patients in the standard CS group, 17,973.6 ± 7,272.5 mg, ($P<0.0001$).

Panels C and D show time-changes of anti-desmoglein 1 and anti-desmoglein 3 antibody ELISA values, respectively. After an initial decrease in anti-desmoglein 1 and 3 antibodies from the start of treatment to month 6 in both treatment groups, a re-increase in anti-desmoglein 1 and 3 antibodies was observed from month 12 to month 24, in patients assigned to the standard corticosteroid group, whereas anti-desmoglein 1 and 3 antibodies remained at very low levels until the end of the study in patients assigned to the rituximab group.

The dashed line represents the cut-off values proposed by the manufacturer for anti-desmoglein 1 and 3 antibodies.

Figure 3: Evolution of total B lymphocytes and desmoglein-specific B lymphocytes, under treatment.

Evolution of peripheral blood CD19+ B lymphocytes (panel A), and desmoglein 1 (full 1ine) and desmoglein 3 (dashed line) CD19+ IgG+ B lymphocytes (panel B) in pemphigus patients according to treatment regimen (red lines: patients treated with the standard corticosteroid regimen; blue lines: patients treated with rituximab).

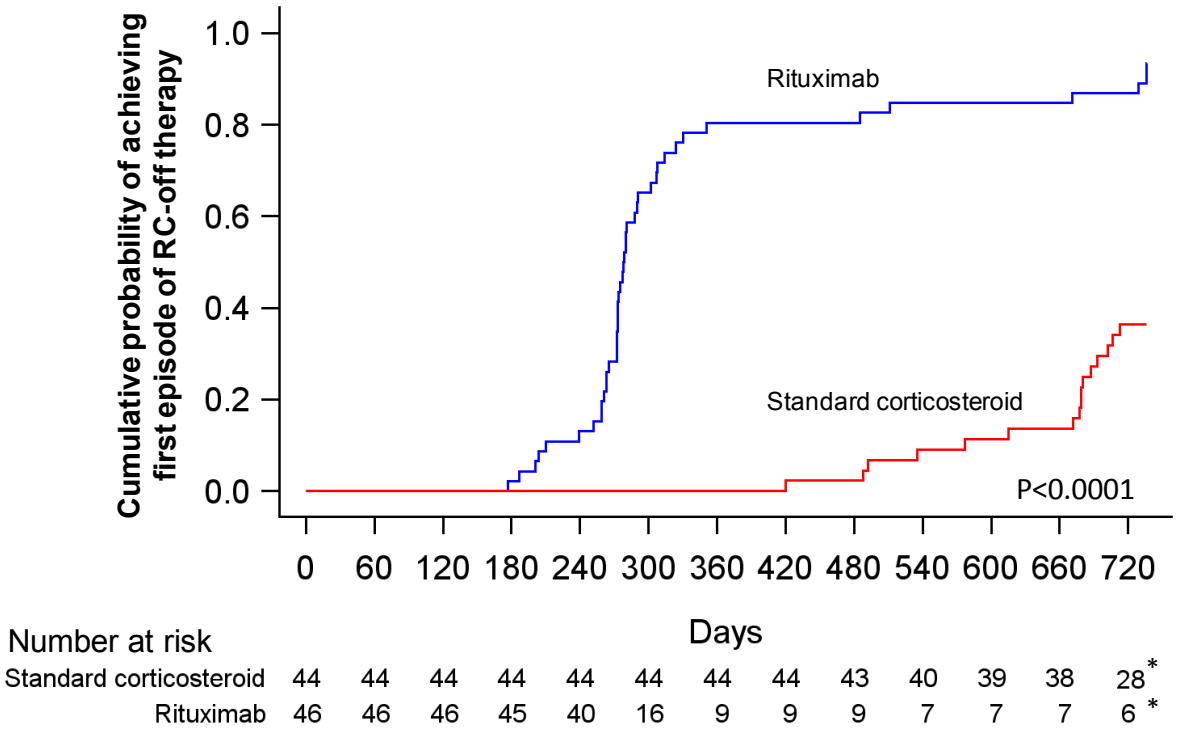
Panel A shows a dramatic decrease of peripheral-blood B-cells in the rituximab group from a median percentage of 8.8% (range 3.3% to 29.9%) at baseline to 0% at day 30. Blood B-lymphocytes remained undetectable until day 240. B-cells then reappeared in 9 out of the18 patients tested, and re-increased up to 5.3 % (range 0.3 to 13 %) at day 360.

After the 2 maintenance infusions of rituximab at months 12 and 18, blood B-lymphocytes remained undetectable until the end of the study. Conversely, no major variation of blood B-lymphocytes was evidenced during the course of patients treated with the standard corticosteroid regimen, from 11.8 % (range 3.3% - 29.9%) at day 0 to 12.7 % (range 3.6% - 40.5 %) at day 360, and 10.5% (range 1.7% - 22.6 %) at the month 24 evaluation.

Panel B shows a dramatic decrease of the respective number of desmoglein 1 and desmoglein 3 IgG+ blood B-lymphocytes in the rituximab group from 35 (range 9 - 88) and 19 (range 6 - 30) cells per million lymphocytes at day 0, to 0 and 0 at day 180, and 2 (range 0-28) and 2 (range 0 to 15) cells per million lymphocytes at day 720.

The respective number of desmoglein 1 and desmoglein 3 specific blood B-lymphocytes from patients assigned to the standard corticosteroid group did not vary during the study period, from 40 (range 5-88) and 26 (range 0-52) per million lymphocytes at baseline to 51 (range 9-118) and 31 (range 4 -100) per million of lymphocytes at day 720.

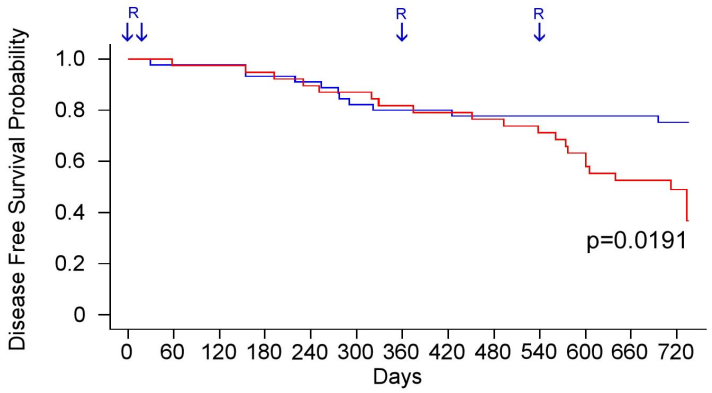
Figure 1



*One patient in the standard CS group and 2 patients in the rituximab group who achieved CR off-therapy during the study, further relapsed during the follow-up, and were no more in CR-off therapy at month 24. Three patients in the rituximab group had their month 24 evaluation a few days after day 720 and were not considered in CR off therapy on this curve.

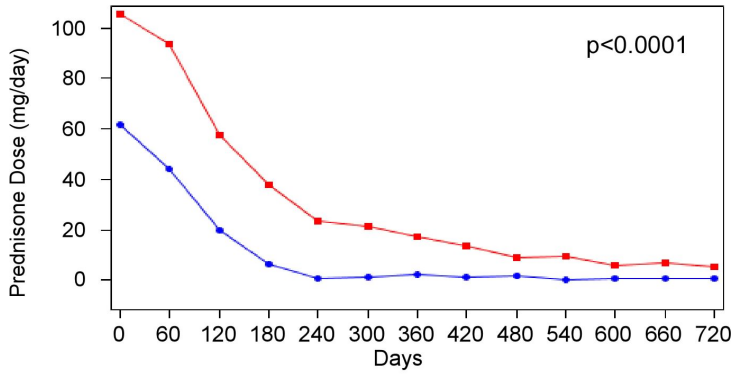
Figure 2

A

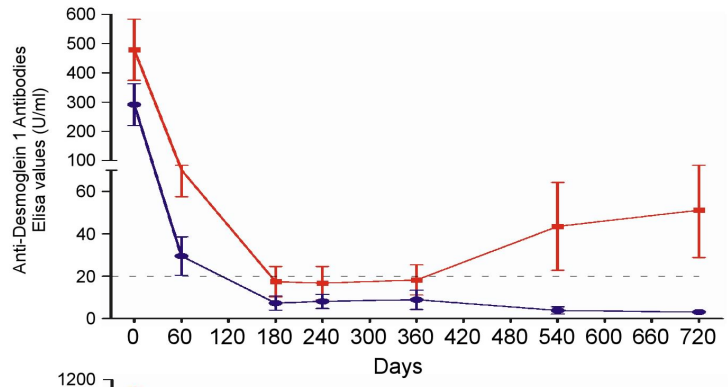


Number at Risk													
Standard Corticosteroid Regimen	43	40	37	36	34	33	31	30	29	27	24	20	10*
Rituximab	45	44	44	42	41	37	36	36	35	35	35	34	22*

B



C



D

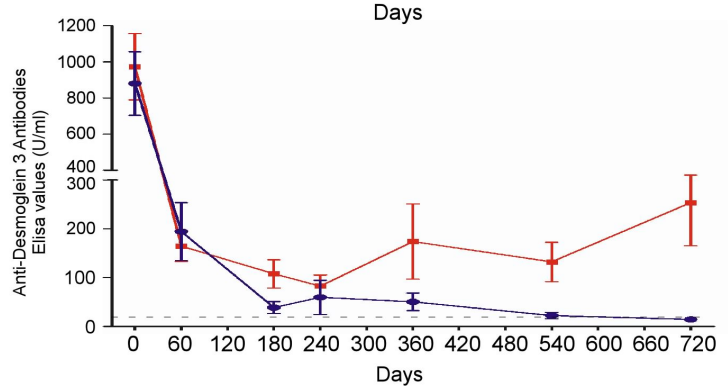
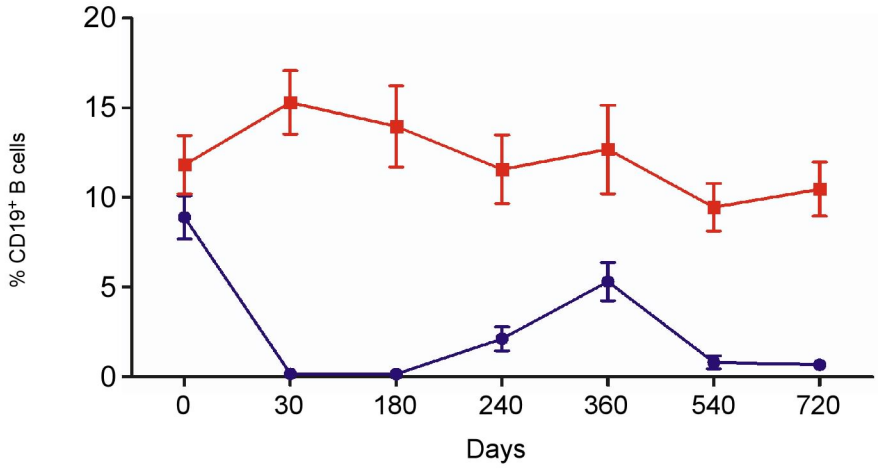
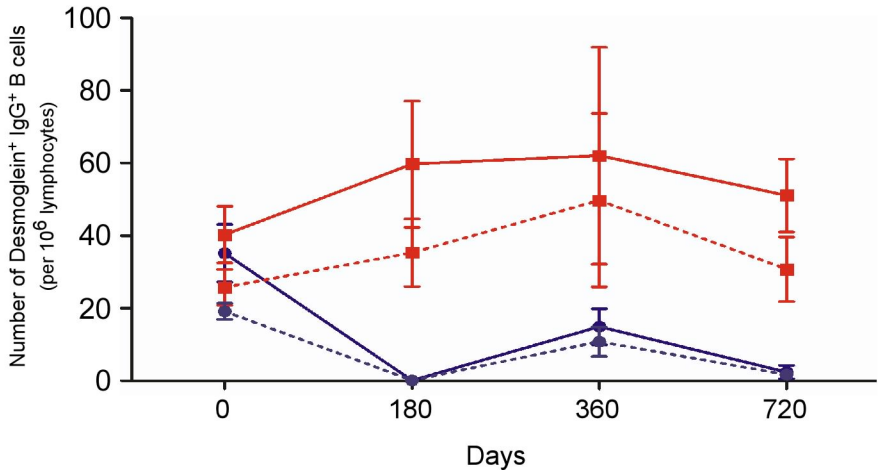


Figure 3

A


20
15
10
5
0
% CD19+ B cells
0
30
180
240
360
540
720
Days

B


100
80
60
40
20
0
Number of Desmoglein+ IgG+ B cells
(per 10^6 lymphocytes)
0
180
360
720
Days