Efficacy of B-Cell Targeted Therapy With Rituximab in Patients With Active Moderate to Severe Graves' Orbitopathy: A Randomized Controlled Study

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Background: Preliminary studies have shown that rituximab (RTX) is effective in the treatment of active Graves' orbitopathy (GO).

Methods: We conducted a double-blind, randomized trial (European Clinical Trials Database [EudraCT] 2007-003910-33) to compare RTX with iv methylprednisolone (ivMP) in patients with active moderate to severe GO. Thirty-two patients were randomized to receive either ivMP (7.5 g) or RTX (2000 or 500 mg). The primary end point was the decrease of the clinical activity score of 2 points or to less than 3 at week 24. Changes of proptosis, lid fissure, diplopia and eye muscle motility, and quality of life score were secondary end points. The number of therapeutic responses, disease reactivation, and surgical procedures required during follow-up and the patients' quality of life were also assessed.

Results: The clinical activity score decreased with both treatments but more after RTX at 16, 20, and 24 weeks ($P < .04$, $P < .02$, $P < .006$, respectively), whether 1000 mg RTX twice or 500 mg RTX once was used (P = NS). At 24 weeks 100% of RTX patients improved compared with 69% after ivMP ($P < .001$). Disease reactivation was never observed in RTX patients but was observed in five after ivMP. Patients treated with RTX scored better motility at 52 weeks in both the right ($P = .014$) and the left eye ($P = .026$). Overall rehabilitative surgical procedures carried out during follow-up (at 76 wk) were 12 in 16 ivMP patients and 5 in 15 RTX patients ($P = .049$).

Conclusions: The results of this trial confirm preliminary reports on a better therapeutic outcome of RTX in active moderate to severe GO, when compared with ivMP, even after a lower RTX dose. The better eye motility outcome, visual functioning of the quality of life assessment, and the reduced number of surgical procedures in patients after RTX seem to suggest a disease-modifying effect of the drug. (***J Clin Endocrinol Metab*** **100: 422–431, 2015)**

Graves' orbitopathy (GO), the most frequent extrathyroidal manifestation of Graves' disease (GD), is a rare disorder that occurs in approximately 2 per 10 000 population per year and in about 25%–40% of patients with GD in a clinically relevant form (1). GO pathogenesis is based on immunological cross-reactivity between thyroid and orbital tissue (2) in which the putative autoantigens, and the mechanisms involved, are still unclear (3). Glucocorticoids have been the therapy of choice in active moderate to severe GO, and treatment effectiveness has

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Abbreviations: CAS, clinical activity score; GD, Graves' disease; GO, Graves' orbitopathy; ivMP, iv methylprednisolone; NOSPECS, no signs or symptoms; only signs, no symptoms; signs only; proptosis; eye muscle involvement; corneal involvement; sight visual acuity reduction; QOL, quality of life; RTX, rituximab; TES, total eye score; TMS, total motility score; TRAb, TSH-R antibody; TSH-R, TSH receptor.

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been reported in as many as 75%–80% of patients (4, 5). In approximately 30% of patients, this therapy is either ineffective or does not prevent disease reactivation (6) and progression toward severe degrees of muscle dysfunction or even dysthyroid optic neuropathy. A very recent multicenter clinical trial of European Group on Graves' Orbitopathy (4) has suggested a treatment schedule with 830 mg iv methylprednisolone (ivMP) administered weekly for 6 weeks followed by 415 mg for another 6 weeks for a cumulative dose of 7.47 g.

B cell depletion with rituximab (RTX), a chimeric mouse-human monoclonal antibody directed against the CD 20 antigen on B lymphocytes has been reported to be effective for the treatment of active moderate to severe GO since 2006 (7, 8). RTX may affect pathogenic TSH receptor (TSH-R) autoantibody by directly targeting B cells in their antigen-presenting cell function (9). Several noncontrolled studies have shown that RTX is potentially useful in the treatment of GO, in particular for the control of the early active, inflammatory phase of the disease (10). Several questions need to be answered before we can consider using RTX in GO. Does RTX modify the course of GO? Given the potentially serious side effects of systemic immunosuppression induced by RTX, can we trustfully use this treatment in patients who are affected by a progressive and often disfiguring disease but also known to be self-limiting and with consequences that can also be satisfactorily corrected by surgery? For these reasons, we have conducted a randomized, double-blind, controlled trial in which patients with active moderate to severe GO were treated with either RTX or ivMP.

Materials and Methods

Patients

The study included adult Caucasian, Asian, Hispanic, or black males and females, aged 18–75 years, smokers and nonsmokers, euthyroid for at least 6–8 weeks (based on the measurement of normal free thyroid hormone concentrations), affected with active GO, defined by a clinical activity score (CAS) of 4 of 10 or greater or 3 of 7 or greater (11), of moderate to severe degree, as defined by the NOSPECS (no signs or symptoms; only signs, no symptoms; signs only; proptosis; eye muscle involvement; corneal involvement; sight visual acuity reduction) score (12, 13). Patients with previous steroid treatment, as long as it had been discontinued for at least 3 months, were included in the study. Main exclusion criteria are shown in Supplemental Table 1.

The study was approved by the institutional review board of our institution and registered with European Clinical Trials Database (EudraCT) number 2007-003910-33. All patients gave written informed consent. Patients underwent clinical endocrinological assessment, biochemical testing [serum free T_4, free T_3, and TSH concentrations were measured using an electrochemiluminescent immunoassay (Roche Diagnostics)], and ophthalmological examination according to a recent European Group on Graves' Orbitopathy consensus statement (13). A complete ophthalmological assessment included Hertel measurements and a study of ductions by the Foerster-Goldman perimeter (14). Soft tissue involvement was graded according to the Color Atlas, available at www.eugogo.eu (15).

Study design

The study was designed to include 60 patients, 30 randomized to receive ivMP and 30 RTX according to the schedule shown in Figure 1. Block randomization with randomly selected block of four patients was planned for this study. Treatment was blind to the patient and to the ophthalmologist. The study was stopped based on an interim analysis on 16+16 patients, as we recorded disease reactivation (5 of 16 patients) in the ivMP group but not in the RTX group.

All ivMP patients were treated with proton pump inhibitors for the prevention of gastric bleeding, with calcium and vitamin D supplementation and bisphosphonate administration for preventing steroid-induced osteoporosis and were administered placebo as premedication as was done in those receiving RTX to maintain masking.

All RTX patients were premedicated with oral paracetamol (1 g), chlorphenamine (10 mg), and 100 mg iv hydrocortisone to prevent commonly occurring hypersensitivity reactions and were infused saline as placebo to maintain masking when they were not administered RTX. We initially infused 1000 mg RTX, twice at a 2-week interval (Figure 1), and 10 placebo infusions, and we subsequently amended the protocol to a single 500-mg RTX and 11 placebo infusions after the first 12 patients. The protocol amendment was based on the recording of complete peripheral B cell depletion after only 100 mg RTX (16).

Clinical outcome measures

Primary end point

A decrease of the CAS 2 or more points or disease inactivation (CAS < 3) at 24 weeks was the primary end point of the study. Active GO patients had a baseline CAS of 3 of 7 or greater or greater than 4 of 10 points.

Secondary end points

Severity signs were secondary end points of the study: reduction of at least 2 NOSPECS classes; decreased proptosis of 2 mm or greater and lid fissure of 3 mm or greater, in at least one eye and improvement of the total eye score (TES), obtained by multiplying each NOSPECS class by the severity grade, as a composite score for clinical response. Improvement of eye motility was assessed as the Gorman score for diplopia of 1 class or greater, eye muscle ductions of 8 degrees or greater by the Foerster-Goldman perimeter, and a total motility score (TMS) (Figure 2A). Also analyzed were disease reactivation at 24 weeks and the number of surgical procedures required after 12 months. Lastly, the patients' quality of life (QOL) was assessed with the validated disease-specific GO-QoL questionnaire (17), by which improvement is scored as an increase of at least 6 points on either GO-QoL scales (functioning and appearance), when compared with baseline.

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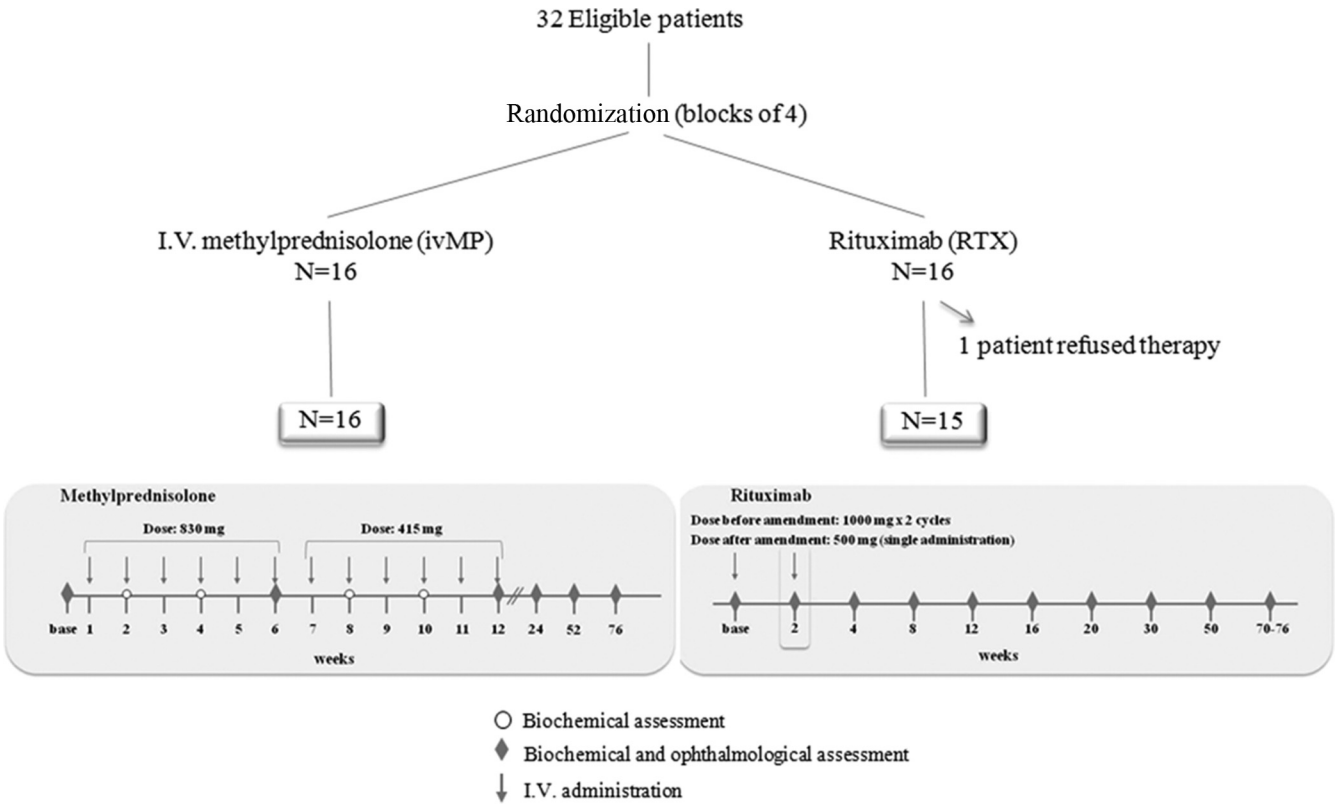
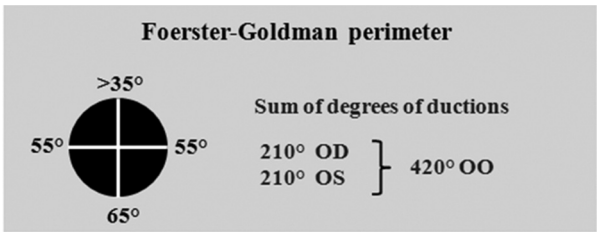


Figure 1. Randomized controlled trial of RTX vs ivMP for the treatment of active moderate to severe GO (EUDRACT 2007–003910–33): study design and schedule of infusion with clinical, biochemical, and immunological assessment and follow-up up to 76 weeks from baseline.

Safety

Exclusion criteria are shown in Supplemental Table 1. RTX patients were monitored for infusion reactions and other potential untoward effects on a monthly basis (serum sickness, infections); ivMP patients were monitored throughout the treatment course for changes of serum liver enzymes, glycemia, and serology for hepatitis B virus and hepatitis C virus. Side effects were classified as major (uncontrolled diabetes, occurrence of major depression, severe infections, and any adverse effect leading to IVGC discontinuation) and minor (worsening of liver function, insomnia, dyspepsia, flushing, myalgia, asthenia, nausea, and development or worsening of diastolic hypertension). Side effects occurring during the therapeutic infusions were usually controlled and monitored by the endocrinologist and did not influence the ophthalmological examination.

A



B

Weeks	0	24	52
IVMP	OD 179.2±8.6 OS 177.3±8.7	OD 178.6±6.7 OS 178.6±7.3	OD 188.2±7.0 OS 192.5±6.8
RTX	OD 187.0±5.8 OS 186.7±6.2	OD 197.3±5.6 OS 191.7±6.5	OD 196.1±5.0 OS 196.4±5.8
P value		OD = 0.019 OS = ns	OD = 0.014 OS = 0.026

Figure 2. Motility assessment in patients with GO after treatment with either ivMP or RTX. A, Calculation of the TMS by assessment of the degrees of ductions by the Foerster-Goldman perimeter. B, Outcome of TMS at 24 and 52 weeks after ivMP and RTX in both the right (OD) and the left (OS) eye (Wilcoxon).

Cytofluorimetry and serology

Lymphocyte subpopulations were analyzed with a standard antibody panel for the following subpopulations: $CD3^+$, $CD3^+4^+$, $CD3^+8^+$, $CD3^+DR^+$, $CD20^+$, $CD19^+5^+$, and $CD56^+16^+3$, at baseline and at each point of follow-up. Serum TSH-R antibodies (TRAbs) were measured as TSH-R binding inhibitory immunoglobulins using a third-generation TRAK human lumitest (Thermofisher AG) at baseline and at each point of follow-up (Figure 1).

Statistical analysis

All values are expressed as mean ± SE or mean ± SD, as specified. Analysis by a Fisher exact test, Wilcoxon test, repeated-measures ANOVA, or Mann-Whitney test was applied, as appropriate, and performed using SPSS 8.0 for Windows (SPSS Inc). Statistical significance was defined as $P < .05$.

Results

Characteristics of the patients

We have randomized 32 patients (26 women, six men, mean ± SD 51 ± 12 y) with active moderate to severe GO

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Table 1. Clinical, Biochemical, Immunological, and Ophthalmological Characteristics of Patients Treated With Either RTX or ivMP at Baseline

	ivMP	RTX	*P* Value
Clinical characteristics			
n	16	15	
Mean age, y (±SD)	50.4 ± 11.4	51.9 ± 13.1	.73
Sex (female)	12	14	.36
Smokers, %	9 (56.2)	10 (66.7)	.41
Thyroid disease			
Graves' hyperthyroidism, %	16 (100)	12 (80)	.20
Primary hypothyroidism, %	0	2 (13.3)	.44
Euthyroid Graves' orbitopathy	0	1 (6.7)	.97
Previous radioiodine	1 (6.2)	2 (13.3)	.95
Previous thyroidectomy	1 (6.2)	1 (6.7)	.96
Current thyroid treatments			
Methimazole	13 (81.2)	9 (60)	.37
Propylthiouracil	1 (6.2)	0	.33
Levothyroxine	2 (12.5)	5 (33.3)	.34
None	0	1 (6.7)	.97
Duration of GO, mo (mean ± SD)	4.6 ± 2.6	4.5 ± 2.9	.77
Previous oral prednisone for GO	3	3	
Previous ivMP for GO	3	3	
Biochemical and immunological characteristics			
TSH, mU/L (mean ± SD)	1.5 ± 2.1	2.3 ± 2.4	.34
FT4, pg/mL (mean ± SD)	13.1 ± 5.6	11.4 ± 3.7	.39
FT3, pg/mL (mean ± SD)	3.9 ± 1.8	3.2 ± 0.6	.13
TRAbs, U/L (mean ± SD)	18.2 ± 21.7	10.7 ± 9.1	.49
TRAbs positive, %	14 (87.5)	13 (86.7)	.94
TPOAbs positive, %	5 (31.2)	3 (20)	.76
TgAbs positive, %	3 (18.7)	2 (13.3)	.68
IgA	211.1 ± 108	214.1 ± 65.5	.93
IgG	965.1 ± 206.5	963.7 ± 150.4	.98
IgM	118.9 ± 73.5	133.9 ± 87.1	.61
CD20, cells/L (mean ± SD)	264.3 ± 115.6	266.5 ± 130.2	.90
CD19, cells/μL, (mean ± SD)	264.9 ± 119.0	270.1 ± 140.1	.98
AST	19.1 ± 6	16.3 ± 3.3	.14
ALT	19.9 ± 12.5	13.9 ± 6	.12
γGT	24.4 ± 20.5	18.5 ± 9.9	.35
Alkaline phosphatase	87.9 ± 42.3	88.6 ± 63.1	.35
Glycemia	84.4 ± 13.8	83.4 ± 14	.84
HbA1c	5.6 ± 0.3	5.6 ± 0.4	.75
Serum creatinine	0.75 ± 0.1	0.8 ± 0.1	.55
Eye symptoms and signs			
CAS	4.7 ± 0.7	4.4 ± 0.7	.49
Proptosis OD	22.8 ± 3.3	23.2 ± 2.5	.74
Proptosis OS	22.5 ± 3.7	23.5 ± 3.5	.46
Lid fissure OD	11.6 ± 2.2	11.4 ± 2.4	.79
Lid fissure OS	11.5 ± 1.8	11.9 ± 2.3	.63
Soft tissue involvement			
Minimal, %	3 (18.7)	4 (26.7)	.92
Moderate, %	10 (62)	10 (66.7)	.80
Marked, %	3 (18.7)	1 (6.7)	.89
Diplopia (Bahn and Gorman score)			
Absent, %	5 (31.2)	5 (33.3)	.90
Intermittent, %	4 (25)	3 (20)	.74
Inconstant, %	3 (18.7)	5 (33.3)	.61
Constant, %	4 (25)	1 (6.7)	.37

Abbreviations: ALT, aminotransferase; AST, aspartate aminotransferase; FT3, free T_3; FT4, free T_4; γ-GT, γ-glutamyl transferase; HbA1c, glycated hemoglobin; OD, right eye; OS, left eye; TgAb, thyroglobulin antibody; TPOAb, thyroperoxidase antibody.

to receive RTX (n = 16) or ivMP (n = 16) (Figure 1). One woman randomized to receive RTX subsequently withdrew from the study. Of the 31 patients included in the study, 28 had GD, two Hashimoto's thyroiditis, and one euthyroid Graves orbitopathy (Table 1). Twenty-three patients were treated with thyonamides (methymazole, n = 22; propylthiouracil, n = 1), and seven patients were on L-T4 therapy for hypothyroidism after radioiodine abla-

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tion (n = 3), total thyroidectomy (n = 2), or spontaneous hypothyroidism (n = 2). Previous iv steroid therapy for active GO was administered to three ivMP patients for a total cumulative dose of 6 g, 8 g, and 4.5 g, respectively, 16, 48, and 12 weeks prior to inclusion in the study and to three RTX patients for a total cumulative dose of 4.5, 1.6, and 5.5 g, respectively, 48, 212, and 20 weeks prior to inclusion in the study. All RTX patients showed peripheral CD20+ cell depletion for 4–24 weeks, independently of the RTX dose used. At 76 weeks the number of B cells in the periphery was still lower than baseline ($P < .004$).

Primary end point

At randomization, two patients had the CAS assessed at the first (3 of 7 points) and 29 at the second examination (4 of 10 points). The CAS decreased to 1.46 ± 0.4 with ivMP and 1.3 ± 0.4 with RTX (P = NS) at 12 weeks to 2.2 ± 0.4 and 0.9 ± 0.3 ($P < .04$) at 16 weeks to 1.6 ± 0.3 and 0.5 ± 0.2 ($P < .01$) at 20 weeks, and 2.3 ± 0.5 and 0.6 ± 3 ($P < .006$) at 24 weeks, respectively (Figure 3A). Although at 24 weeks the number of patients with a decrease of the CAS of 2 points or greater was not different (Table 2; P = NS), disease inactivation (CAS < 3) occurred in 68.7% ivMP vs 100% RTX patients (Fisher, $P = .043$) (Figure 3B). The study has therefore satisfied its primary end point at 24 weeks. Five ivMP patients had relapsing active GO at 24 weeks. A dose-finding analysis has shown no difference in the clinical response related to the therapeutic schedule (ANOVA, P = NS; Figure 3C), despite RTX being administered at a lower dose (500 mg) in about two-thirds of the patients.

Secondary end points

There was no difference in the reduction of two NOSPECS classes treated with ivMP or RTX (P =NS; Table 2). Mean proptosis values did not significantly decrease (Wilcoxon; $P = .67$) in any of the two groups (Mann-Whitney; P = NS). Proptosis reduction of 2 mm or greater was observed in five patients treated with RTX at 52 weeks (Fisher; P = NS, Table 2). Lid fissure reduction of 3 mm or greater was never recorded in ivMP patients but was recorded in two RTX patients at 52 weeks (P =

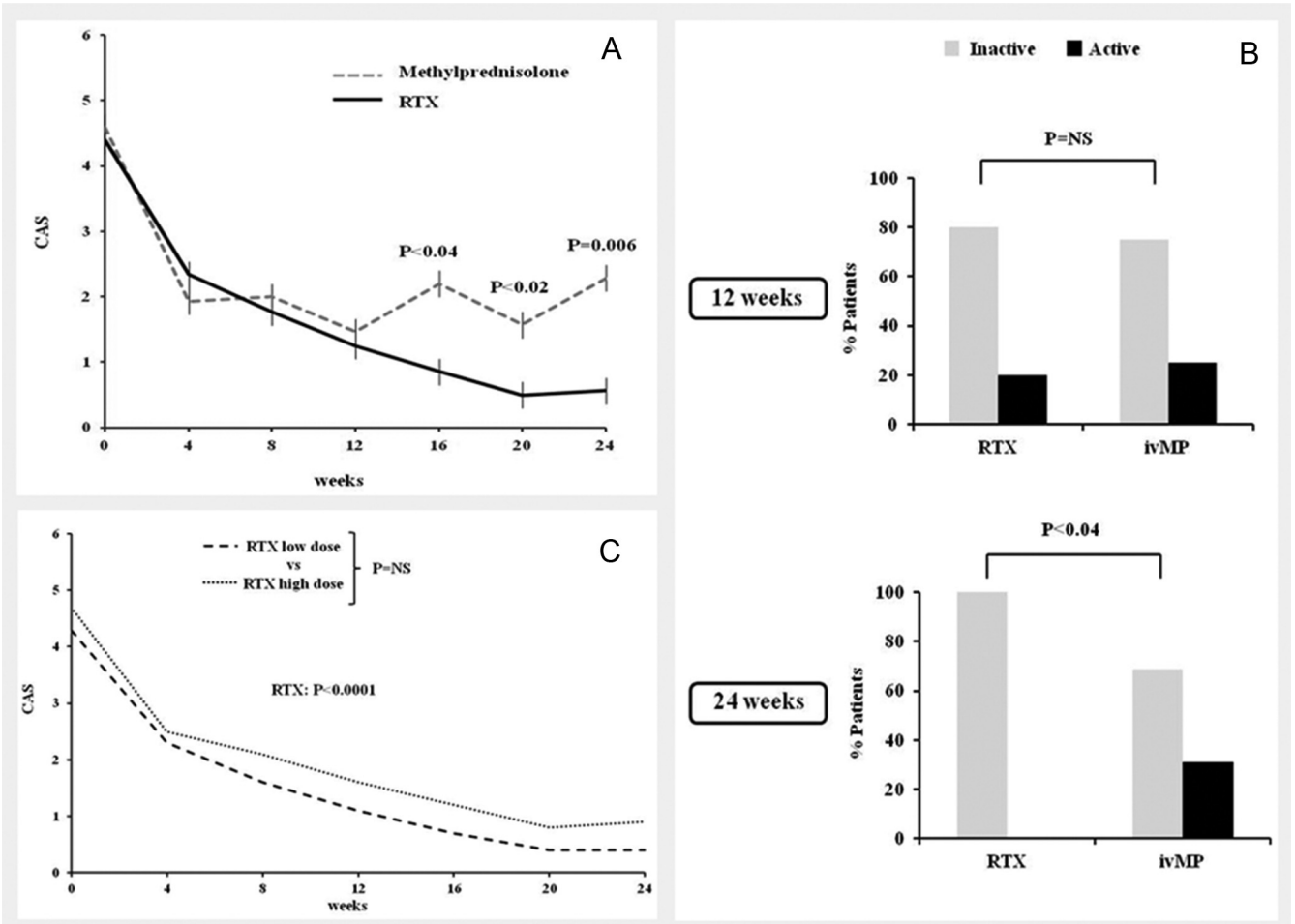

Figure 3. Analysis of the primary end point of the study. A, Changes of the CAS in patients treated with either ivMP or RTX from baseline up to 24 weeks of follow-up (Wilcoxon). B, Outcome of the primary end point of the study after ivMP and RTX at 12 and 24 weeks (Fisher exact test). C, Changes of the CAS after a high dose (2000 mg) of low-dose (500 mg) RTX from baseline up to 24 weeks of follow-up (Mann-Whitney).

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Table 2. Outcome Measures of a Randomized Clinical Trial of ivMP or RTX in Patients With Active Moderate to Severe GO

	Primary End Point		NOSPECS				Proptosis Reduction of ≥ 2 mm		Secondary End Points Severity: Lid Fissure Decrease of at Least 3 mm	
Rand Number	Reduction of CAS ≥2 24 wk	CAS <3 24 wk	Reduction of Two Classes 24 wk	TES ± SD 0 wk	TES ± SD 24 wk	TES ± SD 52 wk	24 wk	52 wk	24 wk	52 wk
ivMP										
1	1	0	0	17	15	13	0	0	0	0
2	1	1	1	18	9	9	0	0	0	0
6	1	1	0	11	12	10	0	0	0	0
8	0	0	0	18	25	6	0	RT	0	RT
9	1	1	0	6	6	0	0	RT	0	RT
12	1	1	0	8	2	6	0	0	0	0
14	0	0	0	12	31	6	0	RT	0	RT
15	1	1	0	8	8	2	0	0	0	0
18	1	1	0	12	10	10	1	1	0	0
19	1	1	0	18	14	6	0	0	0	0
21	0	0	0	8	18	14	0	0	0	0
23	1	1	1	(23)	RT	RT	0	RT	0	RT
25	1	1	0	8	2	0	0	0	0	0
27	1	1	1	14	8	10	0	0	0	0
30	1	1	0	14	14	8	0	0	0	0
32	0	0	0	16	14	14	0	0	0	0
	12/16, 75%	11/16, 68.7%	3/16, 18.8%	12.5 ± 4.3	12.5 ± 7.8	7.6 ± 4.5	1/16,, 6.25%	1/16, 6.25%	0	0
RTX										
3	1	1	0	2	4	6	0	1	0	0
4	1	1	0	4	0	0	0	0	0	0
5	1	1	0	22	21	20	0	0	0	0
7	1	1	0	21	17	17	0	1	0	0
10	1	1	1	(23)	RT	RT	RT	RT	1	RT
13	1	1	0	14	14	14	0	0	0	0
16	1	1	1	18	12	12	0	1	1	1
17	1	1	0	12	10	10	0	0	0	1
20	1	1	0	13	9	13	0	0	0	0
22	1	1	1	14	9	9	0	1	0	0
24	1	1	0	6	2	2	0	0	0	0
26	1	1	0	8	6	6	0	1	0	0
28	1	1	0	11	9	9	0	0	0	0
29	1	1	0	11	11	5	0	0	0	0
31	1	1	0	7	5	5	0	0	0	0
	15/15, 100%	15/15, 100%	3/15, 20%	11.6 ± 6	9.2 ± 5.7	9.1 ± 5.6	0	5/15, 33.3%	2/15, 13.3%	2/15, 13.3%

Abbreviation: Rand, randomization number. An explanation of the numbers and letters used are as follows: 0, no; 1, yes; A, orbital decompression; B, squint surgery; C, lid surgery; RT, retreated. Dashes indicate missing values.

NS). No difference in the Gorman score for diplopia was observed in either group at 24 weeks and 52 weeks (P = NS, Table 1). A decrease in the TES is expected when disease severity improves: mean ± SD TES in ivMP patients decreased significantly at 52 weeks (Wilcoxon, $P < .004$), whereas in RTX patients it decreased significantly at 24 ($P < .005$) and at 52 weeks ($P < .012$; Table 2).

By measuring the total degrees of ductions (TMS) in the four main gaze directions, we were able to record changes of gaze restriction as a consequence of treatment. Although ivMP therapy did not significantly improve gaze restriction, RTX resulted in better motility at 24 weeks for the right eye (197.3 ± 21.6; $P < .019$, Wilcoxon, Table 2) and at 52 weeks for both the right eye (196.1 ± 18.8; $P = .014$) and left eye (196.4 ± 21.7; $P = .026$) (Figure 2B). The outcome of motility at 24 weeks in the right eye was better with RTX when compared with ivMP ($P = .04$, Mann-Whitney).

Five of 16 patients ivMP (31.2%), but none of those treated with RTX, had GO reactivation (Fisher; $P = .043$, Table 2): three at 24 weeks, one at 40 weeks, and one at 52 weeks. One of these patients (Table 2, number 14) developed optic neuropathy, unresponsive to additional steroids but eventually responsive to low-dose RTX (100 mg). Three patients were retreated with ivMP (numbers 8, 9, and 21) and one with low-dose RTX (100 mg) (number 32). Reactivation was not related to thyroid dysfunction because patients were euthyroid at the time of GO reactivation.

Two patients were submitted to surgical orbital decompression during the follow-up period: one woman (Table 2, number 10) with inactive GO after RTX for correction of disfiguring unilateral proptosis at 14 weeks after therapy and one man (Table 2, number 23) after ivMP because of severe corneal involvement. These two patients were excluded from the analysis of the secondary end points. Residual disease signs were evaluated at 76 weeks (Table 2). Ten ivMP patients (62.5%) and three RTX patients (20%; Fisher; $P = .042$) required a surgical procedure, of which six and one, respectively, were surgical orbital decompression. Overall

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Table 2. Continued

Secondary End Points										
Severity										
Motility					Improvement of Go-QoL Scale of at Least 6 Points					
Gorman Score ≥1 Class					Functioning			Appearance		
24 wk	52 w	76 w	GO Relapse	Additional Treatment	24 wk	52 wk	76 wk	24 wk	52 wk	76 wk
0	0	0	0	B	0	0	0	1	0	0
0	0	0	0	0	1	0	0	0	0	1
0	0	0	0	C	0	0	0	0	0	0
0	0	0	1	A, B	0	RT	RT	0	RT	RT
0	0	—	1	0	1	RT	RT	1	RT	RT
0	0	0	0	A	1	1	1	1	0	0
1	—	1	1	0	—	RT	RT	—	RT	RT
1	1	0	0	0	1	1	1	1	1	1
0	0	0	0	B	1	1	0	0	0	0
1	0	0	0	A	—	1	1	—	1	1
0	0	0	1	A	0	0	0	0	0	0
0	0	0	0	A, C	0	0	RT	0	0	RT
0	0	0	0	C	—	1	1	—	1	1
0	0	0	0	0	1	1	0	0	1	1
0	0	0	0	0	1	1	—	1	1	—
0	0	—	1	A	1	0	—	1	1	—
3/16, 18.8%	1/15, 6.7%	1/14, 7.1%	5/16, 31.2%	10/16, 62.5%	8/13, 61.5%	7/13, 53.8%	4/10, 40%	6/13, 46.1%	6/13, 46.1%	5/10, 50%
0	0	1	0	0	0	0	0	0	0	0
0	0	1	0	C	0	1	0	1	1	1
0	0	0	0	B	0	0	—	1	0	—
1	1	1	0	0	0	—	0	1	—	1
0	0	0	0	A, B, C	0	0	1	1	1	1
0	0	0	0	0	0	1	—	0	1	-
1	1	1	0	0	0	1	1	1	1	1
0	0	0	0	0	0	1	0	1	1	1
0	0	0	0	0	1	1	1	0	0	0
0	0	1	0	0	1	1	1	1	1	0
0	0	1	0	0	1	1	1	0	0	0
0	0	0	0	0	0	1	1	1	1	1
1	—	0	0	0	1	1	1	1	1	1
0	1	1	0	0	—	—	—	—	—	—
0	1	1	0	0	1	1	0	0	0	0
3/15, 20%	4/14, 28.5%	8/15, 53.3%	0	3/15, 20%	5/14, 35.7%	10/13, 76.9%	7/12 58.3%	9/14, 64.2%	8/13, 61.5%	7/12, 58.33%

rehabilitative surgical procedures carried out during follow-up (52 and 76 wk) were 12 in 16 ivMP and 5 in 15 RTX patients (Fisher; $P = .049$) (Supplemental Table 2) .

Analysis of the GO-QoL has shown significant improvement only at 12 weeks in ivMP patients in the appearance scale (Wilcoxon; $P = .039$) but never in the visual functioning scale. The impact of RTX therapy on patients' QOL was much more significant because improvement on the appearance scale was recorded at 52 and 76 weeks (Wilcoxon; $P = .027$ and $P = .043$, respectively) and on the visual functioning scale at 12, 52, and 76 weeks ($P = .058$, $P = .01$, and $P = .018$, respectively). At 52 weeks, RTX patients reporting improvement in the functioning scale were more than ivMP patients (Fisher; $P = .05$). At 76 weeks, there were no RTX patients reporting worsening in the functioning scale when compared with ivMP patients (Fisher; $P = .035$; Table 2).

Adverse events

Adverse events occurred in 10 of 16 patients treated with ivMP (62.5%) and 13 of 15 of those treated with RTX (86.6%) (Supplemental Table 3). In detail, we recorded major side effects in three patients after ivMP, consisting in a 10-fold increase of aminotransferases spontaneously normalized at the end of treatment, a 5-fold increase of γ-glutamyl transferase and glycemia and glycosylated hemoglobin, respectively. In two patients treated with RTX, we observed a major infusion reaction, likely a cytokine release syndrome, presenting with a rapid onset of orbital edema and ensuing decrease of vision, controlled with the administration of 100 mg iv hydrocortisone and spontaneously resolved within the next 3 hours. Details of these patients were previously reported (16).

Minor side effects observed in seven ivMP patients were dyspepsia, hypotension, insomnia, and mild mood disorders, which did not require specific treatment (Supplemental Table 3). Mild infusion reactions, characterized by throat itching and nose stuffing, were observed in most RTX patients at first infusion. These symptoms resolved spontaneously in all patients by slowing down RTX infu-

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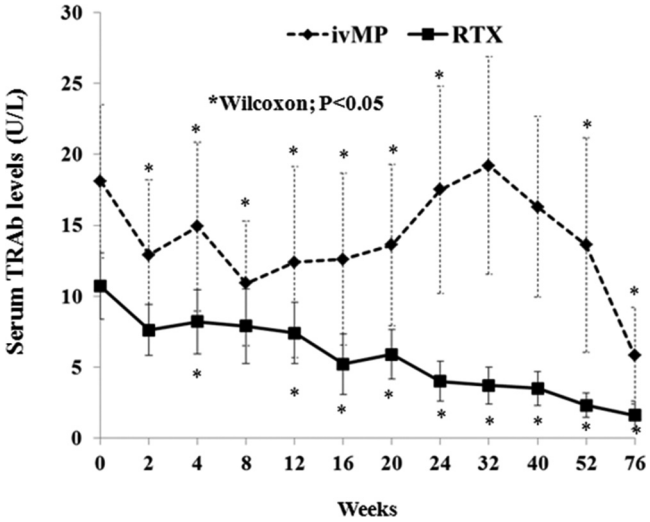

Figure 4. Changes of serum TRAbs in patients with active GO after either ivMP or RTX from baseline up to 76 weeks of follow-up. *, $P < .05$ (Wilcoxon).

sion or by administering 100 mg iv hydrocortisone. One RTX patient had transient hypotension and one patient suffered from a myocardial infarction 8 months after RTX therapy, reportedly unrelated to treatment.

Effect on serum TRAbs

We have measured serum TRAbs at each follow-up visit in both ivMP and RTX patients. A progressive, significant decrease of serum TRAb levels from 18.1 ± 21.6 to 12.4 ± 27.0 at 12 weeks (Wilcoxon; $P = .007$), to 17.5 ± 24.3 at 24 weeks ($P = .05$), to 13.6 ± 25.1 at 52 weeks ($P = .013$), and to 5.8 ± 9.6 at 76 weeks ($P = .036$) was observed after ivMP. Similarly significant was the decrease of serum TRAb levels after RTX, from the baseline value of 10.7 ± 9.1 to 7.4 ± 7.8 at 12 weeks (Wilcoxon; $P = .011$), to 4.0 ± 4.5 at 24 weeks ($P = .005$), to 2.3 ± 2.4 at 52 weeks ($P = .012$), and to 1.6 ± 2.6 at 76 weeks ($P = .018$) (Figure 4). Serum TRAb levels at 32 and 40 weeks after ivMP were not different from baseline, probably in relation to patients' disease reactivation, but at the end of follow-up these TRAb levels were not different from those observed after RTX (Mann-Whitney; P = NS).

Discussion

This randomized controlled study, although limited by the inclusion of a rather low number of patients, shows that RTX is effective in inactivating moderate to severe GO in as many as 100% of patients as compared with 70% of those treated with ivMP, consistent with data previously reported (4, 5). Based on these results, the study satisfies its primary end point. RTX has been used in previous and uncontrolled studies in patients with moderate to severe, and even severe GO, with a reported overall success rate of more than 90% (9). In this study, we have observed that although response to ivMP in GO patients was as effective as RTX at 12 weeks, ie, the duration of steroids administration, during follow-up all the patients treated with RTX remained inactive, whereas about 30% of patients treated with ivMP had disease reactivation. This suggests that RTX acts as a disease-modifying drug when compared with ivMP.

The dose of RTX initially used, according to the study design, was based on previous work in autoimmunity (18). No dose-finding study has previously determined in autoimmune disease which RTX dose is needed to induce B cell depletion and effective treatment. From our previous pilot study (8) and after observing that even minute doses of RTX (100 mg) were effectively depleting B cell from the peripheral blood and orbital tissues (16), we amended the protocol and administered a single 500-mg RTX dose after the first 12 randomized patients. We have performed a dose-finding analysis that confirms that such a dose does induce inactivation of GO as effectively as 1000 mg RTX twice. With a lower RTX dose, patients are exposed to lower risks of potentially severe side effects, such as reactivation of infections (19, 20) or induction of autoimmunity (21).

The analysis of the secondary end points of the study also suggests that RTX modifies the natural course of GO. Parameters assessing changes of eye muscle function are of utmost importance because they condition the final therapeutic outcome in GO patients. We have found that the total motility score, more than the Gorman score, may be an objective measure of improvement of ductions in the patients submitted to immunosuppressive treatment, otherwise not easily quantifiable. This score improves after RTX at 24 and 52 weeks, when compared with ivMP, suggesting that RTX has an impact on the long-term outcome of eye muscle dysfunction, also reflected by the lower number of corrective surgical procedures needed at 76 weeks (discussed below).

The observation of GO reactivation in about 30% of ivMP patients, but in none of those treated with RTX, was the reason for performing an interim analysis on 32 patients and deciding to close the study and proceed to data analysis. The lack of disease reactivation after RTX underscores its effect in modifying the natural course of disease. Steroids also did not prevent disease progression to optic neuropathy and corneal breakdown in two patients. The patients' long-term outcome (76 wk) has shown a greater number of surgical procedures carried out in patients after ivMP, in particular orbital decompression and squint correction. The surgical outcome in GO patients

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has been previously shown in one study (22) to improve with ivMP only in association with total thyroid ablation (thyroidectomy and radioiodine ablation). In this study, treatment has also influenced the patients' QOL. The appearance scale of the QoL questionnaire seemed to be positively influenced by both treatments at 12 weeks, until steroids produced their antiinflammatory effects, but improvement on the visual functioning scale was significant only after RTX, probably in relation to the beneficial effect of RTX on muscle ductions and the long-term surgical outcome.

The reported adverse events confirm the significant prevalence (~10%) of liver dysfunction after ivMP, even when the cumulative dose does not exceed 8 g (6). This finding reinforces the need for mandatory liver function monitoring throughout the therapeutic course. The occurrence of two major infusion reactions after the first RTX dose suggests that this drug should be administered in specialized centers, where all the appropriate rescue measures can be undertaken (23).

As a corollary to the clinical outcome of the study, we also looked at the changes of serum TRAbs in response to either immunosuppressive drugs. Whereas in patients with active GO serum TRAbs have been reported to decrease after ivMP (24), their changes in patients treated with RTX has been attributed to either a direct effect on TSH-R-stimulating antibodies (10, 25) or to remission of hyperthyroidism after long-term antithyroid treatment (26). The present findings show that circulating TRAbs decrease independently of the modality of immunosuppression. In this prospective study, therapy was started in euthyroid patients with the same disease duration, and therefore, data analysis was not influenced by potential confounding factors that may have been present in previous uncontrolled studies (9).

We are aware that in this study there were differences in baseline parameters such as TRAbs, soft tissue involvement, and constant diplopia in the two groups of patients. We do not believe that this is due to failed randomization because patients were included based on their clinical activity score and all had moderate to severe GO of variable degrees, and the recruitment of a greater number of patients in the two arms of treatment would have reduced, rather than increased, these differences. Randomization in this study was designed to analyze the response of disease activity to treatment, and this may result in unmatched severity parameters without reducing the significance of the study.

We have recently become aware of the results of a randomized controlled study conducted by Stan et al (27) in which they did not find RTX effective in treating active GO, when compared with placebo. The study outcome at 24 weeks was the decrease of CAS of 2 or more points and was assessed on 21 patients who concluded the study. Compared with our series of patients, we note differences, at the time of recruitment, in the total number of patients treated (21 vs 31), mean GO duration (11.2 vs 4.5 mo), prior steroid therapy (40% vs 19%), and mean diplopia score (2 vs 3.5). It is possible that differences in the baseline parameters of patients recruited may account for the discrepant results of the two studies.

The inclusion of patients in our study was limited by the strict randomization criteria and the known low disease incidence, and this may be considered a limitation to the interpretation of the results. On the other hand, we believe that the present work is important because it suggests that RTX may be able to modify the natural course of the GO, even as a first-line treatment. An additional strength of this trial is the analysis of the impact of immunosuppression on the residual signs of disease and on the patients' QOL (28, 29), which has rarely been reported in GO in a prospective design (30).

Multicenter trials recruiting greater numbers of patients may be warranted to confirm these results. For now, the results of this study provide important evidence for treating active GO with RTX that so far has been used more and more widely only based on anecdotal reports of efficacy.

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