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Targeting the humoral immune system of patients with rheumatoid arthritis

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Chapter 6

Rituximab fixed retreatment versus on-demand retreatment in refractory rheumatoid arthritis

Comparison of two B-cell depleting treatment strategies

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Submitted

Abstract

Objective

To evaluate efficacy and safety of two B-cell depleting strategies using rituximab in rheumatoid arthritis (RA): fixed retreatment at 24 weeks versus on-demand retreatment at disease relapse.

Methods

Two treatment strategies were compared in a prospective, open-label, non-randomized, two-center study in RA patients refractory to TNF-agents. After initial treatment with 2x1000 mgr rituximab, one group in one center received fixed retreatment at 24 weeks with 1x 1000 mg rituximab, while the other group in the other center received retreatment with the same dose at disease relapse. Clinical efficacy, safety and immunological outcomes were evaluated every 12 weeks during a minimum period of 1 year.

Results

Twenty-eight patients were treated with fixed retreatment versus 20 with on-demand retreatment. At 48 weeks, 64% after fixed retreatment versus 53% after on-demand retreatment achieved an ACR20 (p=0.47), 28% versus 18% an ACR50 (p=0.44) and 4% versus 6% an ACR70 response (p=0.78). With fixed retreatment, initial good responders showed minimal improvement following retreatment: median DAS_{28}-scores decreased from 3.13 [range: 2.76-3.17] to 2.82 [2.50-3.25]; p=0.47). Moderate responders in both groups experienced significant improvement: after fixed retreatment DAS_{28}-scores decreased from 4.62 [range: 3.29-6.10] to 3.17 [2.28-6.15]; p=0.001, after on-demand retreatment from 5.20 [range: 3.82-7.14] to 4.26 [2.66-6.16]; p=0.028). DAS_{28}-scores were lower after fixed retreatment (p=0.09). Non-responders improved only significantly after fixed retreatment (from 5.53 [range: 2.97-6.70] to 3.41 [2.01-5.93]; p=0.028).

Both strategies had comparable incidences of adverse events (86% and 95%) and serious adverse events (18% versus 15%).

Conclusion

Fixed and on-demand retreatment with rituximab showed equal efficacy and safety. Fixed retreatment was more effective in moderate and non-responders to the 1st course.

Introduction

Rituximab, an anti-CD20 monoclonal antibody, has been approved for treatment in patients with rheumatoid arthritis (RA) who have failed treatment with TNF (tumor necrosis factor)-blocking agents[1]. Previous studies demonstrated its safety, efficacy and prevention of radiographic progression[2,3]. Few studies have yet addressed the timing of repeated courses of rituximab. A study in 22 RA patients showed that treatment with repeated courses of rituximab over a 5-year follow-up period was safe and well-tolerated[4]. The safety and efficacy of repeated treatment courses of rituximab was further established in an open-label extension study of 3 large randomized, double-blind studies in RA patients who had experienced adequate responses to previous treatment courses but flared as defined by involvement of at least 8 swollen and tender joints[5]. Clinical efficacy was maintained with subsequent courses of rituximab, comparable to the first rituximab course, without increased additional safety concerns. One study reported that rituximab retreatment was more effective, however, in patients whose disease activity had not completely returned to baseline levels[6]. Therefore, the preferred timing of repeated treatment courses of rituximab is still a matter of debate and, thus far, different B-cell depleting strategies have not been prospectively compared in refractory RA.

The present study compared the efficacy and safety of two B-cell depleting strategies in refractory RA patients, namely fixed retreatment (FR) versus on-demand retreatment (ODR) with rituximab. Clinical outcome, radiographic progression and safety profile were investigated for both treatment strategies.

Patients & Methods

Study design

A prospective, two-center, non-randomized open-label pilot study in patients with severe RA who were treated with an initial course of rituximab (2x 1000 mgr) followed by retreatment with 1x1000 mgr at 24 weeks (‘fixed retreatment’) or at a flare of disease activity (‘on demand’). Patients were allowed to continue their daily or weekly dosages of disease modifying anti-rheumatic drugs (DMARDs) and corticosteroids, however TNF-blocking agents required

an 8-weeks wash-out period. The study was approved by the local Ethics Committee of both centers. Patients enrolled at the Leiden University Medical Center (LUMC) received fixed retreatment and patients enrolled at the University Medical Center Utrecht received on-demand retreatment.
Patients were followed for 24 weeks after a 2nd rituximab course, given as fixed retreatment (FR) or on-demand retreatment (ODR). Patients receiving on-demand retreatment between 24 and 48 weeks had a follow-up between 48 and 72 weeks compared to 48 weeks for the patients receiving fixed retreatment at 24 weeks. Primary and secondary outcomes were compared over a period of 48 weeks. The efficacy of the (fixed or on-demand) 2nd rituximab course was compared over a follow-up period of 24 weeks.

Patients

Patients were eligible when 18 years of age or older, having a clinical diagnosis of RA according to ACR criteria[7] and having failed treatment with combination(s) of DMARDs and/or TNF-blocking agents. Patients were excluded when: life expectancy of less than 6 months, severe uncontrolled infection, irreversible major organ dysfunction, HIV positivity, a positive pregnancy test in women of childbearing age or unwillingness to use adequate contraception for the duration of the study.

Study interventions

Both treatment arms involved an induction phase of 2 infusions of 1000 mg rituximab on day 1 and 14. In the 'fixed retreatment' (FR)-group, patients received retreatment with 1x 1000 mg rituximab at 24 weeks. In the 'on-demand treatment'(ODR)-group, patients received retreatment with 1x 1000 mg rituximab depending on disease activity at the discretion of the treating rheumatologist. Patients with progressive disease after the first rituximab course, defined as less than 15% reduction in disease activity, were excluded for on-demand retreatment. In both treatment groups, tapering of concomitant DMARDs and/or corticosteroids was allowed at the discretion of the treating rheumatologist.

Efficacy parameters

The primary efficacy endpoint was 20% improvement according to ACR response criteria[8]. Secondary efficacy endpoints were response according to EULAR criteria[9], changes in DAS_{28}-scores, physical functioning and radiographic

progression. Patients' clinical disease activity was assessed by research nurses or the treating physicians before intervention and every three months thereafter. Swollen and tender joints were scored, serum markers of inflammation (erythrocyte sedimentation rate and C-reactive protein) were measured, patients' physical functioning was assessed by the Health Assessment Questionnaire (HAQ) and patients' general well-being, pain, stiffness and fatigue was assessed by visual analogue scales. Based on these data, efficacy was determined by ACR response criteria (primary outcome)[7] and the four variable disease activity score of 28 joints (DAS_{28})[10]. Radiographic progression was defined by the change in the total Sharp-vdHeijde score over 1 year[11]. Two trained readers scored radiographs of hands and feet at baseline and at 1 year follow-up. Due to the extensive joint damage in these refractory RA patients, both readers scored radiographs through mutual agreement, while they were blinded for patient's identity, treatment group and chronological sequence of the films.

Safety

The primary safety outcome was categorized according to the WHO common toxicity criteria (CTC)[12]. Adverse events (AEs) were documented by their duration, frequency, severity, cause, their relationship to the study medication, whether it influenced the course of treatment, and whether it required specific therapy. Liver AEs were defined aminotransferase or alkaline phosphatase levels more than 3 times the upper limit of normal, leucopenia as less than $4.5x10^9$/liter, hypoglobulinemia of IgG <7.0 g/L, IgM <0.4 g/L or IgA <0.7 g/L.

Flowcytometric analysis

Whole blood samples were freshly stained for flowcytometric analysis. Cells were incubated with mouse anti-human monoclonal antibodies (mAbs) in PBS/ 1% BSA at 4°C for 30 minutes. The following mAbs were used: anti-CD3-fluorescein-isothiocyanate (FITC); anti-CD16-phycoerythrin (PE); anti-CD56-PE; anti-CD45-peridinin-chlorophyll-protein Complex (PerCP) and anti-CD19-allophycocyanin (APC) (all from Becton Dickinson, San Jose, CA). After incubation TruCount beads (Becton Dickinson, San Jose, CA) were added to the stained cells to obtain absolute numbers after which cells were fixed in 4% paraformaldehyde (LUMC, Leiden, The Netherlands) and analyzed within 24-48 hrs. Stained cells were analyzed with FACScalibur and associated software Cellquest (Becton Dickinson). The detection limit, below which depletion of

CD19+ B-cells was regarded as complete, was set at a frequency of $0.005x10^9$ cells/L.

Measurements of serum antibody titers

Serial serum samples of each patient were analyzed for levels of total immunoglobulins and autoantibodies. Total serum immunoglobulin G (IgG), immunoglobulin M (IgM) and immunoglobulin A (IgA) levels were measured by immunoturbidimetry on the COBAS Integra 400/700/800 (Roche Diagnostics, Indianapolis, Indiana, USA) and nephelometry on the Immage 800 (Beckman Coulter Inc., Fullerton, California, USA) according to the manufacturer's guidelines. Serum levels of anti-cyclic citrullinated protein antibodies (ACPA) of the IgG isotype (ACPA-IgG) were measured using enzyme-linked immunosorbent assay (ELISA) (Immunoscan RA, mark 2; Euro-Diagnostica, Arnhem, The Netherlands), according to the manufacturer's instructions and as previously reported[13]. Serum levels of rheumatoid factor (RF) of the IgM isotype (RF-IgM) were measured using a standardized ELISA, as previously described[14].

Statistical analysis

For primary and secondary efficacy outcomes intention-to-treat analyses were performed using non-parametric Mann-Witney-U tests to compare baseline characteristics, clinical outcome and radiographic progression between maintenance and on-demand treatment group. Chi-square tests were used to compare proportions of patients fulfilling ACR criteria and experiencing any category of adverse events. P-values were considered significant when $p<0.05$.

Results

Study patients

Twenty-eight patients were treated according to the fixed retreatment (FR) protocol and 20 patients according to the on-demand retreatment (ODR) protocol (Figure 1A). After 1 year, 24 patients in the FR-arm completed the study protocol: 1 patient died of progressive lung fibrosis, 2 patients were withdrawn from the study due to an adverse event and 1 patient had progressive disease. In the ODR-arm 17 patients completed the 1-year follow-up: 1 patient was withdrawn because of malignant disease, 1 patient withdrew consent after lack of response to the first treatment course and 1 patient had progressive disease after the

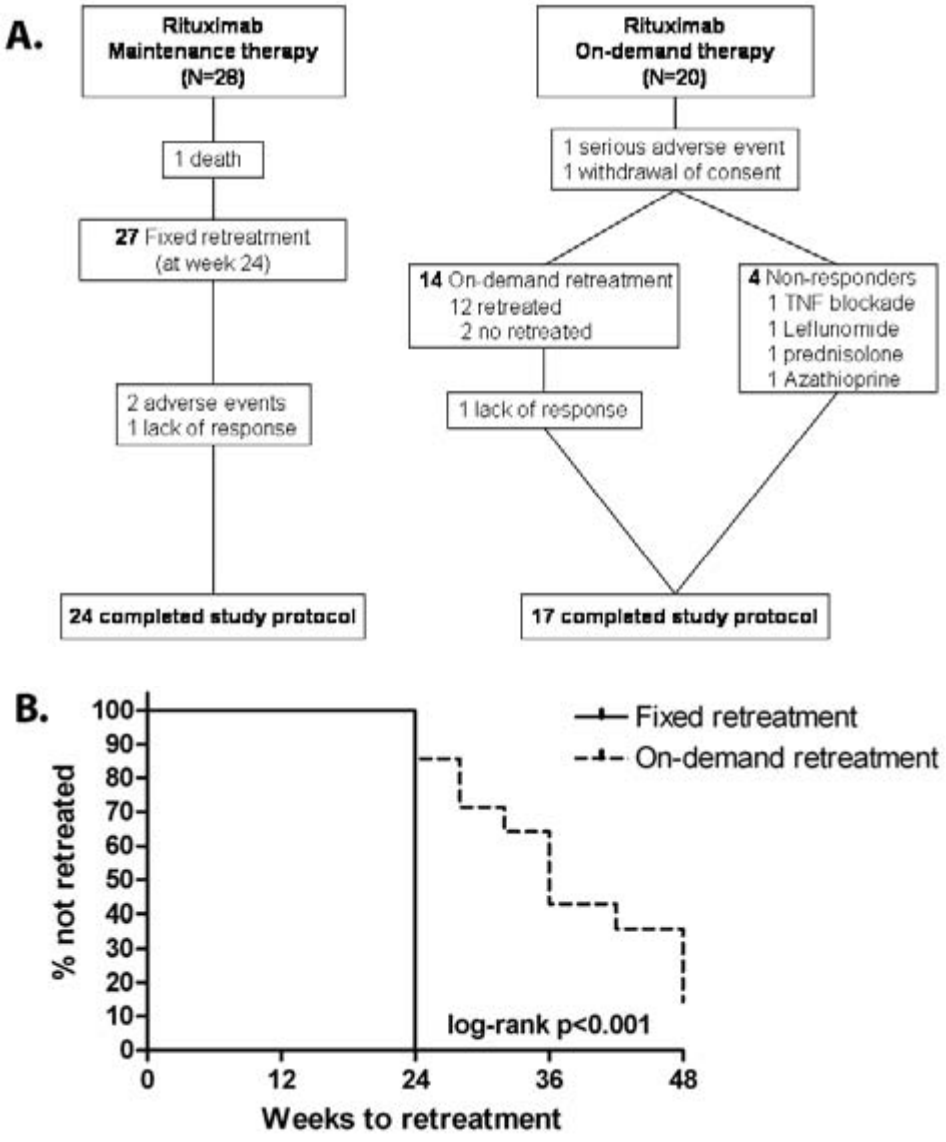

Figure 1 A: Study overview: Twenty-seven out of 28 patients in the maintenance arm were re-treated at the fixed interval of 24 weeks. In the on-demand arm, 18 out of 20 patients were re-treated on demand at a variable intervals: 4 patients were classified as non-responders (therefore receiving treatment with other agents than rituximab), 12 patients were retreated with rituximab and 2 patients had a persisting good response and were therefore not (yet) given retreatment with rituximab. **B:** The interval for initiation a second course of rituximab was 24 weeks for patients receiving fixed retreatment and varying between 24 and 48 weeks for patients receiving on-demand retreatment, as depicted by the Kaplan-Meier curve in which each step indicates a patient receiving on-demand retreatment.

Table 1 Baseline characteristics

	Fixed retreatment (n=28)	**On-demand retreatment (n=20)**	**p-value**
Patients' characteristics			
Age (yrs)	53.7 [32.5-81.5]	58.5 [25.8-83.8]	0.28
Female (%)	71	60	0.41
Smoker			0.07
Current (%)	18	40	
Past (%)	32	45	
Caucasian (%)	93	100	0.24
Employed (%)	32	40	0.14
Disease duration (yrs)	13.2 [1.3-53.2]	13.4 [3.1-50]	0.99
Positive rheumatoid factor (%)	86	95	0.30
Positive ACPA-status* (%)	82	85	0.79
Previous DMARDs (no.)	4 [2-8]	5 [2-11]	0.05
Previous ant-TNF agents (no.)	1 [0-3]	2 [1-3]	0.05
Current DMARDs (no.)	1 [1-2]	1 [0-3]	0.85
RA disease activity			
DAS_{28}-score	6.06 [3.01-7.67]	6.73 [5.17-8.51]	0.09
Tender joints (of 68 joints)	31 [9-58]	28 [10-68]	0.41
Swollen joints (of 66 joints)	9 [2-25]	24 [6-60]	<0.001
ESR (mm/hr)	46 [5-139]	49.5 [12-124]	1.0
CRP (g/L)	25 [2-114]	31 [7-117]	0.81
HAQ-disability	1.63 [0.13-2.88]	1.50 [0.50-2.38]	0.66
Radiographic score*	56.5 [8-245]	52.5 [3-271]	0.57
Visual Analogue Scales			
General well-being* (mm)	51 [2-97]	63.5 [19-78]	0.09
Pain-score (mm)	64 [5-87]	66 [35-95]	0.66
Stiffness-score (mm)	54 [0-96]	70.5 [31-94]	0.03
Fatigue-score (mm)	63 [3-97]	71.5 [1-88]	0.36
Concomitant DMARDs			
Median no. of DMARDs	1 [1-2]	1 [0-3]	0.80
MTX dose (mg/wk)	22.5 [2.5-27.5]	25 [10-30]	0.36
Corticosteroid dose (mg/day)	7.5 [2.5-20]	10 [5-15]	1.0

second treatment course. The time to ODR varied (Figure 1B). Baseline characteristics are summarized in Table 1. Patients in both groups were comparable with respect to most demographic and disease-related data, except for the median number of previously used disease modifying anti-rheumatic drugs (DMARDs), the median number of previously used anti-TNF agents, swollen joint counts, and VAS stiffness, which were all significantly higher in the on-demand group.

Clinical efficacy

ACR20, ACR50 or ACR70 responses over a period of 48 weeks were not significantly different between both strategies (Figure 2A). At 48 weeks 64% versus 53% of patients achieved an ACR20 response (p=0.47), 28% versus 18% an ACR50 response (p=0.44) and 4% versus 5.9% an ACR70 response (p=0.78) in the FR- versus ODR-arm. Also at 12, 24 and 36 weeks ACR responses were not significantly different between both treatment arms. EULAR response categories were comparable between both treatment groups during the complete follow-up (Figure 2B). At 48 weeks, FR led to 28% good responders, 60% moderate responders and 12% non-responders as compared to 20%, 53% and 27%, respectively, in the ODR-group (p=0.48). In addition, after 48 weeks, the change in DAS_{28}-scores was comparable after FR (median change -2.13 [range: -4.10; -0.03]) and ODR (-1.77 [range: -5.78; -0.20]; p=0.51) (Figure 2C). Of interest, maximal improvement in DAS_{28}-score was reached at 36 weeks in the FR-group as compared to 48 weeks in the ODR-group. Radiographic progression was not significantly different between both treatment arms (median 5.0 [range: -11; 30] in the FR-arm versus median 4.0 [range: -13; 23] in the ODR-arm; p=0.33) (Figure 2D). Changes in HAQ-scores were not significantly different throughout the study period (at 48 weeks, median change -0.25 [range: -1.88; 50] in the FR-arm versus median change -0.13 [range: -1.50; 0.63] in the ODR-arm; p=0.48). With respect to concomitant medication at 48 weeks, dosages of methotrexate were significantly lower in the FR-arm (median 20 mg/week [range: 0-27.5] compared to the ODR-arm (median 25 mg/week [range 20-30]; p=0.04). Dosages of corticosteroids were comparable (median 6.25 mg/day [range: 0-20] versus 7.5 mg/day [range: 0-30], respectively; p=0.35).

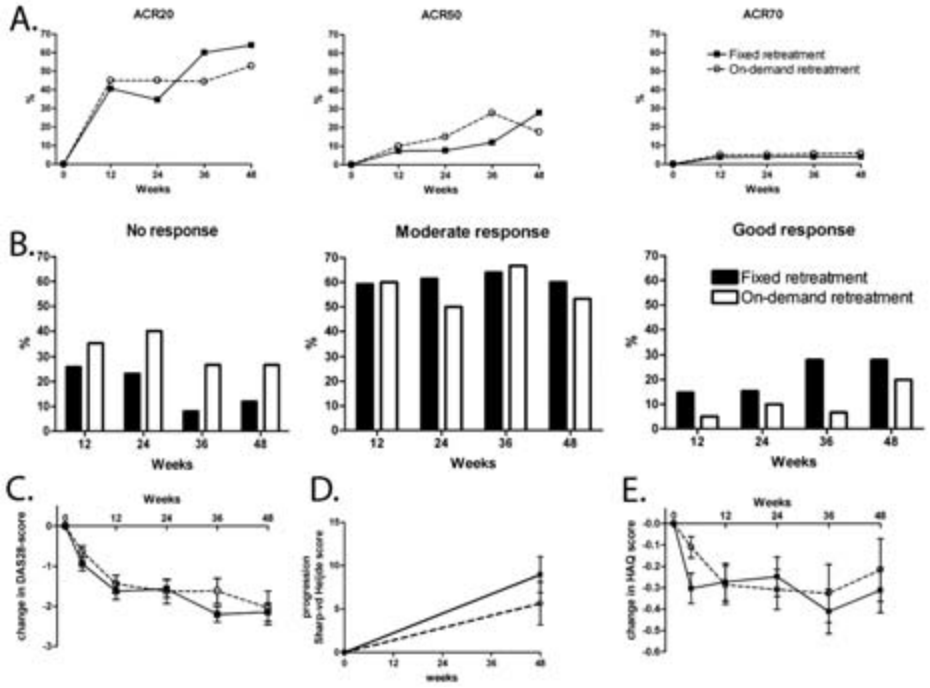

Figure 2 Overview of the clinical endpoints comparing fixed verus on-demand B-cell depletion. **A:** ACR20, 50 and 70 responses were assessed at 12-week intervals. **B**: Response rates according to EULAR response criteria assessed at 12 week intervals. **C:** The change in DAS_{28}-scores. **D:** Radiographig progression as assessed at baseline and 48 weeks by the Sharp-van der Heijde scores. **E:** Change in HAQ-scores.

Clinical efficacy of a 2nd treatment rituximab course

To investigate whether timing of retreatment influenced the efficacy of a 2nd rituximab course, we compared the clinical responses to the 2nd rituximab course over 24 weeks follow-up (Table 2). Twenty-six (out of 28) patients received a 2nd rituximab course in the FR-arm: 2 patients dropped out due to a serious adverse event. Twelve (out of 20) patients received a 2nd rituximab course in the ODR-arm: 1 patient experienced a serious adverse event, 1 patient withdrew consent, 2 patients had a persistent good response to the 1st rituximab course and 4 patients had progressive disease after the 1st rituximab course (Figure 1A). In the efficacy analysis of the 2nd rituximab course, in order to perceive possible over-treatment in the FR-arm or under-treatment in the ODR-arm, patients were separated according to the EULAR response at the time re-treatment was given. Therefore, of 26 patients receiving FR, 4 patients had a

Table 2 Efficacy of 2nd rituximab course given as fixed or on-demand retreatment. Patients were separated for EULAR response 24 weeks after the 1st Rituximab course.

Response to 1st rituximab course	Fixed retreatment (N=26)*	On-demand retreatment (N=12)†	p-value
Good responders	**N=4**	**N=0**	
DAS_{28} at retreatment	3.13 [2.76-3.17]	---	n/a
Lowest DAS_{28} after retreatment°	2.82 [2.50-3.25]	---	n/a
% change in DAS_{28}	6.33 [-9.46; 21.2]	---	n/a
ACR20 (%)	1 (25)	---	n/a
ACR50 (%)	1 (25)	---	n/a
ACR70 (%)	0 (0)	---	n/a
Moderate responders	**N=16**	**N=7**	
DAS_{28} at re-treatment	4.62 [3.29-6.10]	5.20 [3.82-7.14]	0.385
Lowest DAS_{28} after retreatment°	3.17 [2.28-6.15]	4.26 [2.66-6.16]	0.09
% change in DAS_{28}	24.2 [-8.20;49.3]	14.4 [0.65-30.4]	0.122
ACR20 (%)	7 (44)	0 (0)	0.026
ACR50 (%)	2 (13)	0 (0)	
ACR70 (%)	2 (13)	0 (0)	
Non-responders	**N=6**	**N=5**	
DAS_{28} at retreatment	5.53 [2.97-6.70]	5.80 [4.97-7.64]	0.584
Lowest DAS_{28} after retreatment°	3.41 [2.01-5.93]	4.84 [4.71-5.59]	0.121
% change in DAS_{28}	33.4 [11.5-43.8]	18.4 [10.5-26.8]	0.071
ACR20 (%)	2 (33)	1 (20)	0.497
ACR50 (%)	1 (17)	0 (0)	
ACR70 (%)	0 (0)	0 (0)	

* Two patients dropped out due to the occurrence of a serious adverse event.

† Two patients had persisting good response to the 1st rituximab course, 4 patients did not respond to the 1st rituximab course and 2 patients experienced a serious adverse event.

° Lowest DAS_{28}-score achieved at any time during 24 weeks following the 2nd rituximab course

good response at time of retreatment, 16 patients a moderate response and 6 patients no response. Of the 12 patients in the ODR-arm (all of whom had achieved a moderate response after the 1st rituximab course), 7 patients still had a moderate response at the time of retreatment and 5 patients had no response. Importantly, two patients had a persistent good response and retreatment was not (yet) given.

Clinical efficacy after a 2nd rituximab course was assessed by patients' maximal DAS_{28} improvement at any time during 24 weeks follow-up. The median time to maximal improvement was 18 weeks [range: 4-24] after FR and 12 weeks [range: 12-24] after ODR (p=0.88). In good responders, FR led to small reductions in DAS_{28}-scores (median 3.13 [range: 2.76-3.17] to a lowest median score of 2.82 [2.50-3.25]; p=0.47) (Table 2). In moderate responders, significant reduction in DAS_{28}-scores was achieved after FR (median 4.62 [range: 3.29-6.10] to a lowest median score of 3.17 [2.28-6.15]; p=0.001) as well as after ODR (median 5.20 [range: 3.82-7.14] to a lowest median score of 4.26 [2.66-6.16]; p=0.028). Moderate responders achieved significantly better ACR responses after FR (44% ACR20, 13% ACR50 and 13% ACR70) whereas none of the patients met the ACR response criteria after ODR. Consequently, there was a trend to lower DAS_{28}-scores after FR (median 3.17 [range: 2.28-6.15]) compared to ODR (median 4.26 [range: 2.66-6.16]; p=0.09). In non-responders a significant reduction in DAS_{28}-scores was observed in the FR-arm (median 5.53 [range: 2.97-6.70] to a lowest median score of 3.41 [2.01-5.93]; p=0.028), which was not the case in the ODR-arm (median 5.80 [range: 4.97-7.64] to a lowest median score of 4.84 [4.71-5.59]; p=0.11). Subsequently, two patients achieved an ACR20 response and one patient an ACR50 response in the FR-group whereas one patient achieved an ACR20 response in the ODR-group (p=0.50).

Immunological outcome

In both treatment groups, rituximab led to a rapid and complete depletion of CD19+ B-cells in the circulation with detectable reconstitution of B-cells at 24 weeks. After FR as well as ODR with 1x 1000 mgr rituximab, CD19+ B-cells were completely depleted from the circulation (Figure 3A). At 48 weeks, B-cell numbers after FR (mean ± SEM: $32.2x10^6$ ± 20.0 cells per liter) as well after ODR (mean ± SEM: $62.7x10^6$ ± 20.7 cells per liter) were still significantly be-

low baseline levels (respectively, mean ± SEM: $207x10^6$ ± 27.8 cells per liter and mean ± SEM: $161x10^6$ ± 26.8 cells per liter; p<0.001 and p=0.004). Regarding serum levels of autoantibodies, the percentage change in ACPA-IgG (Figure 3C) and RF-IgM levels (Figure 3D) was not different between both treatment groups. At 48 weeks, ACPA-IgG levels decreased to a mean ± SEM 66.5 ± 10.1% of baseline values in the FR-group as compared to 60 ± 13.1% in ODR-group. RF-IgM levels were reduced more profoundly to, respectively, 35.3 ± 6.8% and 42.1 ± 10.5 of baseline values.

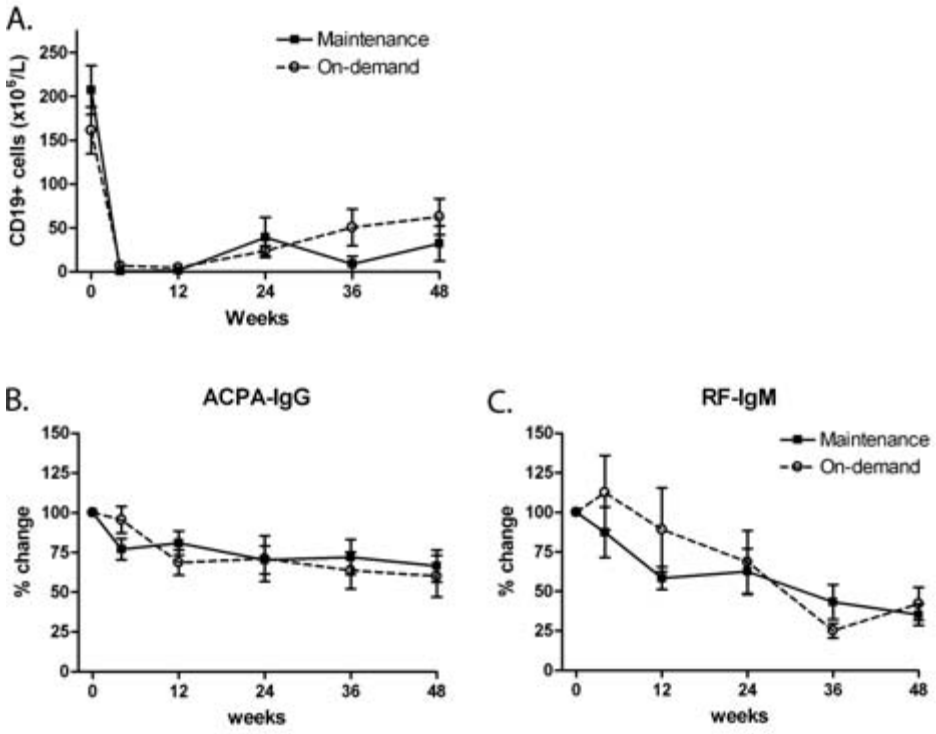

Figure 3 Immunological outcome in both B-cell depleting strategies. **A:** Absolute number of circulating CD19+ cells measured every 12 weeks. Although in the on-demand arm overall B-cell depletion is incomplete, it is noteworthy that in the 12 patients receiving on-demand retreatment with rituximab a complete depletion of circulating CD19+ B-cells was observed. **B:** Percentual change in ACPA-IgG levels. **C:** Percentual change in RF-IgM levels.

Safety outcome

Overall, 86% of the patients in the FR-group compared to 95% in the ODR-group had reported an adverse event (p=0.30) (Table 3). One patient died 8 weeks after initiation of rituximab treatment from respiratory insufficiency due to progression of pre-existent lung fibrosis. Other serious adverse events in the FR-group were a ruptured diverticulitis requiring surgery (leading to withdrawal), infection of prosthesis prolonging hospitalization (leading to withdrawal), acute coronary syndrome (leading to withdrawal), transient ischemic attack and umbilical hernia requiring surgery. Serious adverse events reported in the ODR-group were ovarian cancer (led to withdrawal), progression of proteinuria on the basis of amyloidosis and persistent urinary tract infection requiring prolonged antibiotic treatment. Infusion-related events were seen in 14% of patients in the FR-group and 30% in the ODR-group (p=0.19). All reported symptoms were mild, CTC grade 1 or 2. Leucopenia occurred in 32% in the FR-group and 10% in the ODR-group (p=0.07). Both patients in the ODR-arm experiencing leucopenia did not have lymphocyte, neutrophil or monocyte counts below the lower level of normal (LLN) (respectively, $0.8x10^9$ lymphocytes, $1.6x10^9$ neutrophils or $0.2x10^9$ monocytes per liter). In contrast, 9 patients in the FR-group experienced leucopenia of whom 1 patient was lymphopenic, 2 neutropenic and 1 lympho- and neutropenic. Leucopenia was transient and at 48 weeks all patients had leucocyte counts above LLN and the majority of patients in the FR-group became leucopenic after the 2^{nd} rituximab course. Overall, lymphopenia occurred in 46% of the FR-group and 35% in the ODR-group, which in all but 2 patients did not result in leucopenia. Hypoglobulinemia of IgG occurred in 21% versus 20%, and of IgM in 14% versus 10%, in the FR- and ODR-groups respectively. Patients hypoglobulinemic for IgG had comparable titers below LLN: median 5.70 g/L (range: 5.30-6.40) in the FR-group and 5.79 (range: 3.50-6.80) in the ODR-group (p=1.00). However, IgM hypogamma-globulinemia was significantly more pronounced in the FR-group (median 0.30 g/L [range: 0.20-0.30]) compared to the ODR-group (median 0.35 [range: 0.33-0.36]; p=0.05). The most common infectious-related adverse events were upper airway infection (respectively, 36% versus 60%), nasopharyngitis (respectively, 11% versus 25%) and urinary tract infection (respectively, 14% versus 10%).

Table 3 Summary of adverse events

	Fixed retreatment (n=28)	**On-demand retreatment (n=20)**
Adverse events		
Any adverse event	24 (86)	19 (95)
Serious adverse event	5 (18)	3 (15)
Infusion-related adverse event	4 (14)	6 (30)
Adverse event leading to withdrawal	4 (14)	1 (5)
Death	1 (4)	0 (0)
Adverse events reported (>5%)		
Leucopenia*	9 (32%)	2 (10%)
Lymphopenia†	13 (46%)	7 (35%)
Liver toxicity‡	0 (0%)	2 (10%)
Hypoglobulinemia		
IgG below LLN§	6 (21%)	4 (20%)
IgM below LLN°	4 (14%)	2 (10%)
IgA below LLN¶	0 (0%)	0 (0%)
Upper airway infection	10 (36)	12 (60)
Nasopharyngitis	3 (11)	5 (25)
Urinary tract infection	4 (14)	2 (10)
Eczema	1 (4)	5 (25)
Sinusitus	3 (11)	2 (10)
Infection, gastrointestinal	1 (4)	2 (10)
Itch	0 (0)	4 (20)
Nausea	2 (7)	2 (10)
Cough	0 (0)	3 (15)
Fatigue	3 (11)	0 (0)
Infection, skin	3 (11)	0 (0)
Infection, viral	2 (7)	1 (5)
Nodulosis	3 (11)	0 (0)
Sweating	0 (0)	3 (15)
Fever	0 (0)	2(10)
Pain, musculoskeletal	0 (0)	2 (10)

* As defined by a leucocyte count less than $4x10^9/L$

† As defined by a lymphocyte count less than $0.8 x10^9/L$

‡ As defined by transaminase enzyme levels above 3x upper limit of normal (amino-transferase enzymes >120 units/liter], and for alkaline phosphatase >360 units/liter])

§ Lower level of normal (LLN) for IgG was 7.0 g/L

° Lower level of normal (LLN) for IgM was 0.4 g/L

¶ Lower level of normal (LLN) for IgA was 0.7 g/L

Discussion

The present study compared the efficacy and safety of two B-cell depleting strategies: 'fixed' (FR) versus 'on-demand' (ODR) retreatment with rituximab in RA patients who failed TNF blocking therapy. We demonstrated that over a 48 weeks follow-up period both strategies resulted in comparable efficacy as measured by ACR response, EULAR response, change in DAS_{28}- and HAQ-scores and radiographic progression. In addition, our data indicated that a 2nd rituximab course resulted in significantly better clinical responses in moderate and non-responders when given as a fixed retreatment than on-demand. However, in the FR-arm, 15% of patients with a persistent good response were re-treated with minimal additional effect. With respect to the safety profile, both treatment strategies were comparable. Also both treatment strategies resulted in similar reductions of circulating immunoglobulins and autoantibodies.

To our knowledge, no study has yet compared different B-cell depleting strategies in refractory RA patients. This study is the first to show that fixed retreatment at 24 weeks had similar efficacy and safety as on-demand retreatment (at time of relapse) in RA. Retreatment of patients with Rituximab has thus far only been studied in on-demand fashion[4,5]. In line with these studies, our data showed that a 2nd rituximab course is efficacious and safe in patients who have responded to a 1st course. However, our study provided evidence that a 2nd rituximab course was significantly more effective in moderate and non-responders when given 'early' as fixed retreatment instead of on demand, when disease had flared. Even though patients with on-demand retreatment improved upon a 2nd Rituximab course, DAS_{28}-scores were significantly lower in patients with fixed retreatment, notably in the moderate responders. These results are in keeping with a recent report that retreatment with rituximab was more effective when given at the time response to the previous treatment was waning than when given at the time a full-blown relapse occurred[6]. On the other hand, our study also showed that retreatment at 24 weeks is not always useful: patients with a good response only showed minimal further improvement in DAS_{28}-scores which were not clinical significant (reduction of 0.6 points in DAS_{28}-score[9]). The latter was the case in 4 out of 26 patients (15%) in the FR-arm. Taken together, these data suggested that early retreatment with rituximab is warranted for patients with a moderate or no response at 24 weeks after a 1st

rituximab course. However, in patients with a good response, retreatment may not be necessary or can be postponed.

At the time our study was designed no data had been published on the optimal retreatment dose and frequency, although the commonly used dose is now similar to initial dosage schemes, i.e. 2x 1000 mgr. Dose-ranging studies have only been performed in RA patients receiving a first treatment course with rituximab[3]. The optimal dosage of subsequent courses is still unclear and likely dependent on the timing of retreatment. In the present study we chose to retreat patients with 1x 1000 mgr of rituximab on the premise that, notably in FR-group, the number of CD19+, including CD20+, B-cells present was very low at 24 weeks[15,16]. Our data showed that the latter held true for patient groups both in the FR- as well as ODR-arm. In both treatment strategies, the 2nd course of 1x 1000 mgr rituximab was sufficient to completely deplete CD19+ B-cells. Our data indicate there is a need for studies comparing different dosages of rituximab as retreatment courses.

Despite the relatively small number of patients, the frequency and severity of adverse event were comparable between both B-cell depleting strategies. However, the present study revealed a trend towards an increased frequency of leucopenia in the FR-group. B-cells make up 1-3% of the circulating leucocytes, making it unlikely that rituximab-mediated B-cell depletion by itself was responsible for the observed leucopenia. A recent study in lymphoma patients linked neutropenia to B-cell recovery dependent upon homeostasis of stromal-derived factor-1 (SDF-1), which is known to have a central role in neutrophil emigration from bone marrow and early B-cell lymphopoiesis[17]. Although the exact mechanism is still unclear, leucopenia was transient in all patients and did not lead to increased incidence of infectious-related adverse events in the present study.

The current study was an open-label pilot study to evaluate the safety, feasibility and efficacy of two B-cell depleting strategies. Limitations of our study were the small number of patients and the non-randomized two-center study design. Therefore, patient groups in each treatment arm were not completely comparable. Patients who received ODR tended to have worse disease characteristics, including a higher number of swollen joints and trends to worse stiffness and

higher usage of previous anti-rheumatic treatments (DMARDS and TNF-blocking agents). However, patients had comparable radiographic damage, disease duration and comparable EULAR responses after the 1st rituximab course.

In conclusion, our data demonstrated that over a 1 year period fixed and on-demand retreatment with rituximab were equally safe and effective. Retreatment with rituximab was more effective in moderate and non-responders when given at 24 weeks instead of awaiting disease flare or excluding patients from retreatment. Further randomized studies are needed, however, to evaluate different repeat treatment strategies.

References

1. Cohen SB *et al.* Rituximab for rheumatoid arthritis refractory to anti-tumor necrosis factor therapy: Results of a multicenter, randomized, double-blind, placebo-controlled, phase III trial evaluating primary efficacy and safety at twenty-four weeks. *Arthritis Rheum* **54**: 2793-806 (2006)
2. Edwards JC *et al.* Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *N Engl J Med* **350**: 2572-81 (2004)
3. Emery P *et al.* The efficacy and safety of rituximab in patients with active rheumatoid arthritis despite methotrexate treatment: results of a phase IIB randomized, double-blind, placebo-controlled, dose-ranging trial. *Arthritis Rheum* **54**: 1390-1400 (2006)
4. Popa C *et al*. Repeated B lymphocyte depletion with rituximab in rheumatoid arthritis over 7 yrs. *Rheumatology (Oxford)* **46**: 626-30 (2007)
5. Keystone E *et al.* Safety and efficacy of additional courses of rituximab in patients with active rheumatoid arthritis: An open-label extension analysis. *Arthritis Rheum* **56**: 3896-908 (2007)
6. Mease P *et al.* Predicting outcome of second course of rituximab for rheumatoid arthritis. EULAR SAT0019, 2007. Ref Type: Abstract
7. Arnett FC *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* **31**: 315-24 (1988)
8. Felson DT *et al.* American College of Rheumatology. Preliminary definition of improvement in rheumatoid arthritis. *Arthritis Rheum* **38**: 727-35 (1995)
9. van Gestel AM *et al.* Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. *Arthritis Rheum* **39**: 34-40 (1996)
10. van der Heijde DM *et al*. Development of a disease activity score based on judgment in clinical practice by rheumatologists. *J Rheumatol* **20**: 579-81 (1993)
11. van der Heijde DM *et al*. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. *Lancet* **1**: 1036-8 (1989)
12. WHO common toxicity criteria. 2007. Ref Type: Internet Communication
13. Teng YK *et al.* Immunohistochemical analysis as a means to predict responsiveness to rituximab treatment. *Arthritis Rheum* **56**: 3909-18 (2007)
14. van Esch WJ *et al.* Differential requirements for induction of total immunoglobulin and physiological rheumatoid factor production by human peripheral blood B-cells. *Clin Exp Immunol* **123**: 496-504 (2001)

15. Leandro MJ *et al*. Reconstitution of peripheral blood B-cells after depletion with rituximab in patients with rheumatoid arthritis. *Arthritis Rheum* **54**: 613-20 (2006)
16. Roll P *et al*. Regeneration of B-cell subsets after transient B-cell depletion using anti-CD20 antibodies in rheumatoid arthritis. *Arthritis Rheum* **54**: 2377-86 (2006)
17. Dunleavy K *et al.* B-cell recovery following rituximab-based therapy is associated with perturbations in stromal derived factor-1 and granulocyte homeostasis. *Blood* **106**: 795-802 (2005)